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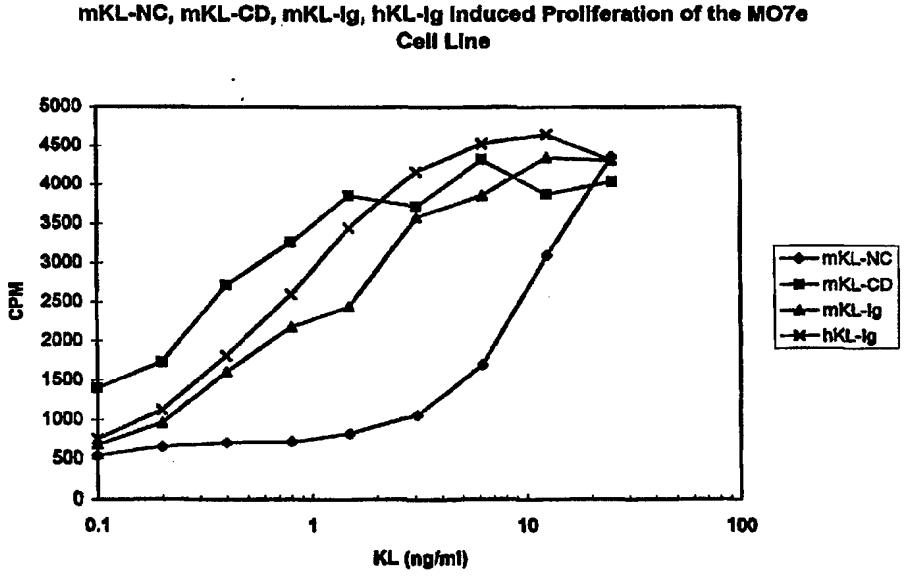

(57) Abstract

A modified form of KL, the ligand for the c-kit proto-oncogene, has been prepared wherein the protein is stabilized by an intermolecular covalent linkage. The protein can be prepared by expression of a recombinant protein wich is dissolved in denaturant and refolded under conditions resulting in a disulfide linked dimer. Examples demonstrate the purification and characterization of this disulfide-linked cysteine dimer kit ligand (KL-CD) which contains at least one intermolecular disulfide bond and has at least ten-fold greater activity in promoting cell proliferation than native, non-covalently linked KL, as measured in *in vitro* assays. The figure shows the proliferative activity of murine KL-NC, murine KL-CD, murine KL-Ig fusion, and human KL-Ig fusion on an MO7e cell line.

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COVALENT DIMERS OF KIT LIGAND AND FLT-3/FLK-2 LIGAND

BACKGROUND OF THE INVENTION

Kit ligand (KL) is a growth and differentiation factor for an assortment of cell types, and is known to be a ligand for the c-kit proto-oncogene. KL was initially identified based on a variety of biological activities and has therefore been referred to by different names, including Stem Cell Factor, Mast Cell Growth Factor, and more recently Steel Factor, in recognition of the gene locus in the mouse which encodes KL, as described by Anderson, et al., (1990) Cell 63, 235-243; Huang, E., et al. (1990) Cell 63, 225-233; Martin, et al. (1990) Cell 63, 203-211; Nocka, et al., (1990) EMBO J. 9, 3287-3294; Williams, et al., (1990) Cell 63, 167-174; Zsebo, et al. (1990) Cell 63, 195-201; Zsebo, K.M., et al., (1990) Cell 63, 213-214.

The ability of KL to promote the proliferation of a variety of cell types indicates that KL is useful as a therapeutic in a variety of clinical indications where enhanced hematopoietic recovery would be beneficial. For example, KL stimulates the survival and proliferation of immature hematopoietic stem cells and progenitor cells, as reported by deVries, et al. (1991) J. Exp. Med. 173, 1205; McKniece, et al., (1991) Exp. Hematol. 19, 226-231; Metcalf, et al., Proc. Natl. Acad. Sci. USA 88, 6239-6243; Nocka, et al. (1990) EMBO J. 9, 3287-3294. Thus, KL could be used for the *ex vivo* expansion of stem cells and progenitors from donor bone marrow prior to transplantation, as proposed in U.S. Patent No. 5,199,942 to Gillis. KL also acts on erythroid progenitors, and in combination with erythropoietin, drives their differentiation, as reported by Nocka, et al., (1990). This property should make KL useful in treating anemias such as that associated with patients having Diamond Blackfan Syndrome, described by Alter, et al., (1992) Blood 80, 3000-3008. KL is also a potent growth factor for megakaryocytic progenitors and in combination with late acting thrombopoietic factors such as IL-6, stimulates megakaryocytic differentiation, as reported by Briddell (1991), Blood 78, 904-911. KL could thus be useful in stimulating megakaryocyte proliferation and platelet production in thrombocytopenic patients Andrews, et al., (1992) Blood 80, 920-927; Hunt, et al., (1992) Blood 80, 904-911. KL has also been shown to be a potent cytokine in the mobilization of stem cells from the bone marrow to the peripheral blood and, in combination with G-CSF, results in significantly greater numbers of progenitor cells than are mobilized through other treatments, as reported by Andrews, et al., (1992) Blood 80, 920-927; Molineux, et al., (1991) Blood 78, 961; Andrews, et al., (1992) Blood 80, 2715; Briddell, et al., (1993) Blood 82, 1720-1723. Stem cells and progenitors that have first been mobilized and then collected from the peripheral blood have been shown by Juttner, et al. (1992) Int. J. Cell Cloning 10, 160, to be useful either

alone or in combination with a bone marrow transplant to speed hematopoietic recovery post radio/chemotherapy.

While KL has many properties which make it a potentially useful therapeutic, KL also acts as a mast cell priming factor and secretagogue, promoting the release of mast cell-derived proinflammatory mediators which can lead not only to local tissue inflammation but more dangerously, to systemic anaphylaxis, as observed by Coleman, et al., (1993) J. Immunol. 150, 556-562; Columbo, et al., (1992) J. Immunol. 149, 599-608; and Nakajima, et al., (1992) Biochem. Biophys. Res. Comm. 183, 1076-1083. The mast cell activating property of KL has been shown to limit the therapeutic potential of native KL. In phase one clinical trials by Amgen of KL administered to patients undergoing chemotherapy, a significant number of patients experienced anaphylactic episodes in response to the KL therapy, mandating their removal from the KL treatment. Crawford, et al., (1993) Proc. Am. Soc. Clin. Oncol. 12, 135; Demetri, et al., (1993) Proc. Am. Soc. Clin. Oncol. 12, 142. Patients that received lower doses of KL, less than 25 μg/kg/day, exhibited minimal side effects; however, at this dose range, KL alone provides little benefit in terms of hematopoietic recovery or peripheral blood progenitor mobilization.

KL, and the receptor to which it binds, the proto-oncogene c-kit, are considered to be members of the Platelet Derived Growth Factor (PDGF) family. Members of this family have several common features, including the structure of the ligands, described by Nocka, et al., (1990); Flanagan, et al., (1991) Cell 64, 1125-1135; Huang, et al., (1992); Bazan (1991) Cell 65, 9-10; Huang, et al., (1990) and the structure and mechanism of action of the receptors, as described by Williams, et al., (1989) Science, 243, 1564-70.

The synthesis and expression of KL is similar to other members of the PDGF family, particularly colony stimulating factor-I (CSF-1 or Macrophage-CSF (M-CSF)) Kawasaki, E.S., et al., (1985) Science 230, 291-296; Wong, G.G., et al., (1987) Science 235, 1504-1508, and the recently identified ligand for the Flt-3/Flt-2 receptor Lyman, et al., (1993) Cell 75, 1157-1167. M-CSF is synthesized from multiple mRNA transcripts that encode for transmembrane proteins, but which lead to either a predominant cell surface bound CSF-1 molecule due to the lack of one proteolytic cleavage site, or to a soluble, proteolytically cleaved CSF-1. Rettenmeier, C.W., Roussel, M.F. (1988) Mol. Cell. Biol. 8, 5026-5034. Similarly, there are at least two naturally occurring forms of KL that arise due to alternative mRNA splicing, as reported by Anderson, et al. (1990), Flanagan, et al., (1991), and Huang, et al., (1992). Both forms are first synthesized as transmembrane proteins. The most abundant form (KL-1) gives rise to a protein of 45 kDa which has a proteolytic cleavage site at amino acids 164-165 (Martin, et al., (1990)), and is readily cleaved to give rise to a soluble protein subunit of 30-35 kDa (Huang, et al., (1992)). The second form of KL (KL-2) is derived from a message in which exon 6, encoding the proteolytic cleavage site, has been spliced out (Anderson,

et al., (1990); Flanagan, J.G., et al., (1991). Without this site a less efficient proteolytic site is used, and the majority of KL-2 remains as a cell surface protein (Flanagan, et al., (1991); Huang, et al, (1992)).

KL does not contain an intermolecular disulfide bond; although it occurs as a dimer when isolated, the units are held together solely by non-covalent interactions (Nocka, et al., (1990); Arakawa, (1991) J. Biol. Chem. 266, 18942-18948. Thus, as analyzed by gel filtration chromatography, soluble KL (KL-1) migrates as a dimer of approximately 60 kDa, when glycosylated or 40 kDa when not glycosylated. However, when analyzed by SDS-PAGE under reducing or non-reducing conditions, native KL migrates with an apparent molecular weight of a monomer, between 30 and 35 kDa when glycosylated or between 18 and 20 kDa when not glycosylated. It is unknown whether membrane associated KL, KL-2, exists in a dimeric state.

cDNA's encoding human, mouse, and rat KL have been cloned and expressed in mammalian, yeast and bacterial cells, as disclosed in PCT/US91/04274 by Immunex Corporation and PCT/US90/05548 by Amgen, Inc. The recombinant KL proteins have biological activity that is comparable to naturally occurring KL of the appropriate species. The protein has been shown to have intrachain disulfide bonds between cysteines at amino acid residues 4 and 89 and at residues 43 and 138, as described by Immunex and Amgen. As described by Amgen, when human KL was expressed as an insoluble protein in *E. coli* and refolded into active protein, the predominant form of the protein was a properly oxidized protein having a molecular weight of between 18,000 and 20,000 Da as determined under non-reducing conditions. A 37,000 Da protein was also observed under non-reduced conditions; however, no mention of biological activity was made. As reported by Immunex, mutants that were truncated to amino acid 138, that had the first two amino acids removed from the N-terminus, and that were missing the fifth glycosylation site were all active.

Recombinant KL from human and rodent preparations has been found to be as effective as the native molecules when assessed in a variety of *in vitro* hematopoietic assays. Lu, et al., (1991) J. Biol. Chem. 266, 8102-8107; Martin, et al., (1990); McKniece, et al., (1991). The therapeutic potential of recombinant KL was suggested by its efficacy in several pre-clinical animal models. For example, administration of KL to rodents at dosages of 100 and 200 µg/kg/day led to significant increases in platelets, reticulocytes, and white blood cells, and to a dramatic increase in the number of circulating progenitor cells, as reported by Molineux, et al., (1991); Bodine, et al., (1993) Blood 82, 445-455. Primate studies demonstrated a similar effect of KL on the hematopoietic system, as reported by Andrews, et al., (1991) Blood 78, 1975-1980. An important study in baboons demonstrated a dose-response effect of KL which mirrored effects seen in later clinical trials; KL had little effect on the hematopoietic system at dosages of between 10 and 25 µg/kg/day, but significant effect at between 100 and 200 µg/kg/day, as described by Andrews, et al.,

(1992) Blood 82, 920-927. Additionally, in a mouse irradiation model, pre-treatment with KL rescued most of the animals exposed to a dose of radiation that was lethal to untreated animals, as described by Zsebo, et al. (1992) Blood 89, 9464-9468.

Although animal models suggested efficacy of KL in stimulating hematopoiesis, when assessed in a clinical trial for its ability to promote the mobilization of stem cells and myeloid progenitors from the bone marrow to the peripheral blood in patients who had received chemotherapy, significant toxicity, manifested as anaphylactic episodes or localized tissue inflammation, occurred in many patients in response to KL, as reported by Crawford, et al., (1993); Demetri, et al., (1993). This toxicity was attributed to the mast cell priming-degranulating activity of KL, and occurred at dosages of 50 µg/kg/day or greater, below the dosage required for effective stem cell mobilization. Thus, native KL can be considered to possess an unfavorable "P:A" (cell proliferation:mast cell activation) ratio.

The ligand of the receptor FLT-3/FLK-2 ("FL") shares a number of biochemical functional properties with KL (Lyman et al., (1993)). Like KL, naturally occurring forms of FL exist as non-covalently associated dimers. Also, FL is synthesized as a transmembrane protein which undergoes a cleavage event to yield a soluble protein. The receptor for FL was originally found to be expressed on immature hematopoietic progenitors/stem cells in the mouse (W. Matthew et al., Cell, 65, pp. 1143-52 (1991)). Thus, like KL, FL is active, especially in combination with other cytokines, in stimulating early progenitor cells and has the potential to have a direct effect on stem cells. Unlike KL, however, FL is not active on erythroid progenitors (Hannum et al., Nature, 368, pp. 643-48 (1994)). It does however act synergistically with GM-CSF to enhance granulocyte and macrophage colony growth.

FL has not been reported to have any activity on mast cells and thus would not be expected to cause mast cell-related side effects when used in a clinical setting. The specific activity of FL is rather low in both proliferation and colony-forming assays and is comparable to that of native KL (Lyman et al., Blood, 83, pp. 2795-801 (1994)). Because of the non-covalent nature of native FL and the overall structural similarity between FL and KL, covalent dimers of FL are also expected to have increased proliferative activity.

It is therefore an object of the present invention to provide a modified form of KL which shows increased potency in mediating cell proliferation *in vitro*, but no increase in its ability to promote mast cell priming.

It is a further object of the present invention to provide methods for making and using a modified KL having a more favorable P:A ratio which can stimulate hematopoietic recovery or stem cell/progenitor cell mobilization with less toxicity than native KL due to mast cell activation.

SUMMARY OF THE INVENTION

The invention provides modified, biologically active kit ligands and FLT-3/FLK-2 ligands which are dimerized by intermolecular covalent crosslinks. These dimers possess surprisingly increased cell proliferation activity as compared to their naturally occurring, non-covalently dimerized counterparts. In addition, the kit ligand dimers of this invention do not possess any significantly greater mast cell degranulation activity over their naturally occurring counterpart. These properties make the dimers of this invention more useful in a therapeutic setting.

The invention also provides recombinant DNA molecules that are useful for producing the various dimers of this invention, as well as host cells transformed or transfected with those DNA molecules.

The invention further provides methods for producing the disclosed dimers from various recombinant expression products.

Finally, the invention provides pharmaceutical compositions and methods which utilize these dimers for the stimulation of cell proliferation, particularly hemopoietic cells and, in the case of kit ligand dimers, for desensitizing mast cells in a patient who will be treated with a therapeutic dose of either covalent or non-covalent kit ligand.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an alignment of the amino acid sequence of the soluble form of Kit Ligand (amino acids 1 to 165) from human (SEQ ID NO.:2), murine (SEQ ID NO.:4), and rat species (SEQ ID NO.:5).

Figure 2A is the elution profile from a first C18 column; where non-covalently linked mKL (KL-NC) elutes at approximately 38% n-propanol, KL-CD elutes at approximately 45% n-propanol, and a third peak containing a different form of KL-CD with very low activity elutes after the biologically active KL-CD peak.

Figure 2B is a photograph of SDS PAGE under reducing and non-reducing conditions of KL-CD and KL-NC eluted from the C18 column shown in Figure 2A.

Figure 2C is a graph of KL bioactivity, CPM tritium incorporated versus nL fraction added, for peak A (dark squares), peak B (open squares), and peak C (dark diamonds) of Figure 2A, as measured by the ability to promote proliferation of the cell line MO7e.

Figure 3A is a photograph of SDS-PAGE of KL-CD and KL-NC refolded from KL-NC in the presence or absence of 2M guanidine HCl.

Figure 3B is a chromatogram of a C18 reverse phase HPLC separation of refolded material as shown in Figure 3A.

Figure 3C is a graph of KL bioactivity of fractions from the chromatogram shown in Figure 3b.

Figure 4 is a graph of KL bioactivity showing proliferation of MO7e in response to purified KL-NC (open squares), KL-CD (dark diamonds), and human KL (dark squares).

Figure 5 is a graph of enhancement of mast cell degranulation by purified KL-NC (open squares) and KL-CD (dark squares) in the presence of anti-TNP and TNP-BSA.

Figure 6 is a graph of enhancement of mast cell degranulation (percent release above antigen alone) versus time (minutes) for KL.

Figure 7 is a bar graph of the desensitization of mast cell degranulation using antigen (dark bars) alone or after prior exposure to KL (open bars).

Figure 8 is a graph of the colony forming unit-granulocyte/macrophage activity of KL-NC (dashed line) and KL-CD (solid line) on colonies of bone marrow cells.

Figure 9 is a graph of the proliferative activity of murine KL-NC (diamonds), murine KL-CD (squares), murine KL-Ig fusion (triangles) and human KL-Ig fusion (x's) on an MO7e cell line.

Figure 10 is a graph of the effect of KL-Ig (triangles), KL-CD (squares) and KL-NC (diamonds) on the priming of IgE induced degranulation of mast cells.

Figure 11 depicts the HPLC profile of endoproteinase Asp-N cleaved, reduced and non-reduced KL-NC (panels A and B), active KL-CD (panels C and D) and inactive KL-CD (panels E and F).

Figure 12, depicts the HPLC profiles of recombinant human KL before and after reduction and refolding (panel A), the MO7e growth stimulating activity of various fractions from those HPLC separations (panel B) and an SDS-PAGE of the various HPLC fractions (panel C).

Figure 13 is a graph comparing cell mobilization and expansion of progenitors from the marrow to the spleen induced by subcutaneous injections of KL-NC or KL-CD.

Figure 14 is a graph comparing cell mobilization and expansion of progenitors from the marrow to the spleen (hatched bars) and peripheral blood (dark bars) induced by a constant infusion of KL-NC or KL-CD.

Figure 15 is a graph comparing cell mobilization and expansion of progenitors from the marrow to the spleen (hatched bars) and peripheral blood (dark bars) induced by KL-NC or KL-Ig.

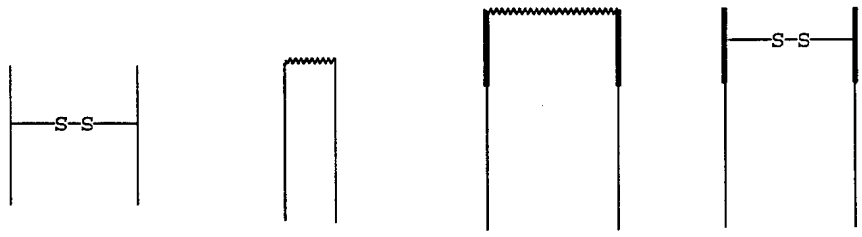
DETAILED DESCRIPTION OF THE INVENTION

As described herein, it has been discovered that it is possible to prepare a covalently crosslinked biologically active dimer of kit ligand, which is significantly more active than native KL in its ability to stimulate cell proliferation, without concomitantly increased mast cell degranulation activity.

As used herein, the term "covalently crosslinked biologically active dimer of kit ligand" refers to molecules which consist of two monomers, each comprising kit ligand amino acids, which are covalently bonded to one another via at least one intrachain molecular bond, preferably a disulfide bond, that links the side group of an amino acid in the first monomer to the side group of an amino acid in the second monomer. That term also encompasses molecules which comprise the above-described two monomers connected to one another via a stretch of amino acids that binds to the C-terminus of one monomer and the N-terminus of the second monomer. All of these molecules encompassed by this term are capable of binding to c-kit and promoting cell proliferation in the assays described herein.

The term "kit ligand amino acids" refers to at least amino acids 1-138 of naturally occurring kit ligand from any species, as well as mutants thereof characterized by conservative amino acid substitutions, by the addition of up to four extra cysteines, by the substitution of up to four amino acids with cysteines, by the substitution of one or two cysteines with another amino acid, preferably serine, or by the deletion of one or two cysteines.

The following picture exemplifies each of these types of covalently crosslinked dimers of kit ligand according to the invention:



The first two modified KLs above are referred to generally herein as KL-CD, for KL-covalent dimer. Non-covalently linked KL is referred to herein as KL-NC. The last two modified

KLs above are referred to generally herein as KL-(X), where (X) is an abbreviation for the source of the non-KL amino acid sequences (e.g., KL-Ig).

Similarly, the invention provides biologically active covalently crosslinked dimers of the FLT-3/FLK-2 ligand, which are identical to the above-described kit ligand dimers, but comprise FLT-3/FLK-2 ligand amino acids substituted for the kit ligand amino acids. Excluded from this invention are dimers comprising immunoglobulin amino acids as non-FLT-3/FLK-2 ligand amino acids. Such molecules have previously been described in international patent application WO 94/28391.

The term "FLT-3/FLK-2 ligand amino acids", as used herein, means at least amino acids 1 to 135 of any naturally occurring molecule that binds to the receptor called FLT-3 or FLK-2, as well as mutants thereof characterized by conservative amino acid substitutions, by the addition of up to four extra cysteines, by the substitution of up to four amino acids with cysteines, by the substitution of one or two cysteines with another amino acid, preferably serine, or by the deletion of one or two cysteines.

Nucleotide and Amino Acid Sequences of Kit Ligand

The nucleotide sequence for murine kit ligand is shown in SEQ ID NO.:3; the corresponding amino acid sequence is shown in SEQ ID NO.:4. The nucleotide sequence for human kit ligand is shown in SEQ ID NO.:1; the corresponding human amino acid sequence is shown in SEQ ID NO.:2.

There is appreciable conservation at the primary sequence level among KL from the different species, in particular in the number and location of the cysteine residues, as shown by Figure 1. Human (SEQ ID NO.:2), murine (SEQ ID NO.:4) and rat (SEQ ID NO.:5) molecules are highly conserved at the amino acid level, with 79%, 80%, and 92% identity between human and mouse, human and rat, and mouse and rat, respectively. Furthermore, the number and location of the cysteines are absolutely conserved. Accordingly, the results shown in the examples for murine KL-CD and human CD may be extrapolated to other mammalian species of KL.

It is not necessary, and in fact is not preferred, to utilize the nucleotide sequence encoding the full length KL; in a preferred embodiment, the sequence encodes only the soluble portion of KL -- at least the first 138 amino acids, more preferably the first 162, 164, or 165 amino acids. Conservative substitutions, additions, and deletions based on differences in amino acid sequence using sequence alignment, as well as based on similarities in structure, charge, and chemistry, can be made to yield a functionally equivalent KL, referred to herein as KL, unless specifically noted otherwise.

1. Formation of KL-CD having at least one interchain disulfide bond in place of at least one of the intrachain disulfide.

A preferred form of KL-CD described in Example 1 of this application contains at least one intermolecular disulfide bond in place of at least one of the intramolecular disulfide bonds found in KL-NC. This form of KL-CD has been demonstrated to be ten fold more potent than KL-NC in its ability to support the proliferation of a number of different types of cells. However, the mast cell priming/activating property of KL is not increased in the KL-CD molecule.

KL-CD can be obtained by expression of the nucleotide sequence encoding KL in an appropriate procaryotic or eucaryotic expression system, for example, *E. coli*, followed by unfolding in urea and refolding in a basic buffer having a pH of between about 8 and 9.

Polypeptides other than those consisting exclusively of naturally occurring kit ligand amino acids which can be used to form dimers of this invention are described below.

2. Deletion of one of the four cysteines in naturally occurring KL

A mutant KL dimer could also be formed as described above, where there is only one intrachain disulfide bond, by deletion of one of the cysteine residues not required for intrachain disulfide bond in KL-CD nor essential for biological activity.

The biologically active form of KL-CD, described in Example 1 and characterized in terms of its disulfide linkages in Example 8, might consist of one intramolecular disulfide bond in each KL monomer, and intermolecular disulfide bonds linking the other two cysteine residues. Mutation of one of the cysteine residues of KL, particularly one which is involved in the intermolecular disulfide bonds in the KL-CD molecule described in Example 1, to another amino acid such as serine, might result in the formation of a molecule with an interchain disulfide between the same cysteine residue on the two monomers plus a single intrachain disulfide on each of the two monomers. Since only one intramolecular disulfide bond could form from such a mutation, this mutation could result in a much greater yield of active KL-CD-like molecule, as compared to the yield of active KL-CD as seen in Example 1.

Specifically, covalent dimers of KL with desirable biological properties can be formed by the substitution of one of the other four cysteines (4, 43, 89 and 138), most preferably 43 or 138, with another amino acid, preferably serine. KL with any three of the four cysteines could fold into KL-CD with similar or different properties to that formed from KL with four cysteines.

In addition to the above-described homodimers of mutant KL, the invention also encompasses heterodimers of mutant KL. More specifically, the invention includes disulfide linked dimers formed by combination of two different subunits of KL, each one having a different cysteine

replaced by another amino acid, preferably serine. The cysteine replacement in the monomers that make up the heterodimer would preferably be at the particular cysteines that normally participate in intramolecular disulfide bonds so as to force the formation of intermolecular disulfide bonds. For example, because a normal intramolecular bond forms between Cys43 and Cys138, a preferred heterodimer consists of one monomer with Cys43 replaced by a serine (e.g., SEQ ID NO: 34) and one monomer with Cys138 replaced by a serine (e.g., SEQ ID NO: 36). Another preferred heterodimer consists of a Cys4->Ser monomer and a Cys89->Ser monomer.

Dimerization of the two heteromonomers may be achieved through a number of techniques. For example, the monomers may be separately expressed in bacteria, isolated by the same methods utilized for KL-CD, denatured with urea or guanidine and then combined with one another and allowed to renature and form disulfide bonds under standard conditions. The ratio of one heteromonomer to the other may be altered to achieve optimal formation of the active disulfide-linked dimer. Determining this optimal ratio may be achieved by assaying the refolded material by HPLC, biological activity and SDS-PAGE under both reducing and non-reducing conditions. Verification that the desired intramolecular disulfide bonds have been formed may be obtained by mapping disulfide-linked peptide fragments of the dimer.

An alternate method of forming these heterodimers is the coexpression of the two heteromonomers in the same host on the same or different vectors. This may be achieved through sequential or simultaneous transfection of any appropriate host cell with the vector(s), each vector containing a selectable marker. If multiple vectors are used, different selectable markers are used on each vector. If a mammalian host is used, the monomers may be expressed through the use of a signal peptide fused to at least amino acids 1-138 of KL containing the appropriate Cys replacement. Alternatively, the full-length KL containing the native transmembrane domain and the appropriate Cys replacement may be expressed. This latter construct may facilitate dimer formation, proper processing and trafficking through the cell to the cell surface. The heterodimer would then be processed by endogenous proteases and released into the extracellular medium.

3. Addition of one or more cysteines to naturally occurring KL

One or more cysteines can be added to KL to allow formation of additional intrachain or interchain disulfide bonds. For example, a fifth or additional cysteine(s) can be introduced into the cDNA to facilitate the formation of interchain disulfide bonds in addition to the two native intrachain disulfides. This interchain disulfide can be placed within a region of KL analogous to that of M-CSF. M-CSF contains an interchain disulfide formed between the cysteines at amino acid 31 in the two monomers, which is within the region where the two monomers are juxtaposed. Thus, mutation of an amino acid residue to cysteine within amino acids 18 to 30 of KL would be expected

to generate a form of KL-CD with properties similar to that of the KL-CD described in example 1. More specifically, an additional amino acid such as another cysteine can be introduced between residues 25 and 26 since these residues must be interrupted by a single amino acid "space" in order to align the sequences of KL and M-CSF. (Bazan, F. (1991) Cell 65, 9-10). Alternatively, a location for an additional cysteine designed to yield an intermolecular disulfide bond can be determined through the elucidation of the disulfide pairs in the KL-CD described in Example 8.

More preferably, an additional cysteine is added to KL by a Cys->Tyr substitution at amino acid 26 or by inserting a cysteine between Tyr_{26} and Met_{27}. Most preferably these additional cysteine-containing monomers are represented by SEQ ID NO.:18 and SEQ ID NO.:20.

4. Formation of KL fusion protein dimers

Covalent dimers or higher order multimers of KL with increased biological activity can be produced through the fusion of monomers comprising non-KL amino acid sequences as well as KL amino acid sequences. The non-KL amino acid sequences in each monomer preferably form interchain covalent interactions with each other, thus eliminating the need for interchain crosslinking to occur between the KL amino acid sequences in each monomer. These covalent dimers display similar activity to the other covalent dimers described in this application. Preferably the non-KL amino acid sequences are derived from immunoglobulins, C1q or C4bp binding protein. Most preferably the non-KL amino acid sequences are derived from immunoglobulins.

An example of this is with immunoglobulin (Ig) Fc domain fusion proteins which have been used for the expression of a number of proteins as dimeric molecules, as described by Lindsley, P.S., et ak (1991) J. Exp. Med. 174, 561-569.

KL fusion proteins may also be generated for use in *ex vivo* cell culture, where the KL fusion proteins are immobilized to a solid substrate. This can be accomplished through the use of KL-Fc fusion proteins bound to Protein A beads. This can also be accomplished by the addition of a collagen binding domain to KL directly or via an Fc bridge so that KL can be coupled to collagen beads or coated substrates.

Methods for making soluble dimeric protein which is expressed on the host cell surface as a chimeric fusion protein incorporating the extracellular portion of the protein with the stem region of C4b binding protein (C4bp) are described in U.S. Serial No. 08/118,366 filed August 8, 1993, the teachings of which are incorporated herein. The protein can be cleaved from the multimeric surface protein to yield soluble protein. A dimeric protein can also be produced by expression of a plasmid vector incorporating the segments of the gene encoding placental alkaline phosphatase (PAP) adjacent to sequence encoding the extracellular region of the KL cDNA amino acids 1-164 or 165,

and a lipid anchor, the cleavage site for a phospholipase, as described in PCT/US92/01867, the teachings of which are incorporated herein.

Another type of fusion dimer within the scope of this invention is a protein comprising the formula KL-linker-KL, where "KL" are kit ligand amino acids and "linker" is a string of between 3 and 50 amino acids which acts as the covalent crosslink between the two KL monomers. This kit ligand dimer may be directly expressed in recombinant systems without the need for forming interchain covalent bonds.

The linker in this construct may comprise any combination of amino acids which are resistant to proteolysis and which reduce aggregation of the final product. The choice of amino acid combinations which possess these properties is within the skill of the art and has been described in WO 94/12520. Preferably, these linkers will comprise from 1 to 9 repeating units of the sequence Gly-Gly-Gly-Gly-Ser (e.g., amino acids 168-172, 173-177, 178-182 and 183-187 of SEQ ID NO:32).

5. Formation of Chemically coupled KL dimers.

Chemical methods, not involving peptide or disulfide bond formation, which form a covalent bond between monomers may also be used to make KL dimers. Methods using a variety of commercially available bifunctional reagents that are available which crosslink proteins, for example, via free amino groups, can be utilized. The reagent DSS from Pierce Chemical Co., would be suitable for this purpose and its use is well known to those skilled in the art. Alternatively, the reagent BASED (Pierce) is a photoreactive crosslinker which reacts non-specifically and could be useful for crosslinking near the dimer interface of KL-NC.

a. Expression and isolation of KL-CD

According to one embodiment, dimers are made by (a) transforming or transfecting a suitable host cell with a recombinant DNA molecule characterized by a nucleic acid sequence encoding a polypeptide comprising kit ligand amino acid sequences; (b) incubating said host cell under conditions which cause expression of said polypeptide; (c) isolating said polypeptide from contaminant polypeptides which do not contain said kit ligand amino acids; (d) optionally employing crosslinking means to convert at least a portion of said isolated polypeptide molecules into a covalently crosslinked dimer of kit ligand; and (e) separating said covalently crosslinked dimer of kit ligand from monomeric forms of kit ligand and from inactive dimers of kit ligand. Preferably, the method employs nucleic acid sequences selected from SEQ ID NOS: 1, 3, 7, 11, 13, 17, 19, 27, 31, 33, or 35.

The optional employment of crosslinking means is necessary when the kit ligand amino acid-containing monomers do not spontaneously form covalent dimers. This step will usually be necessary, except when the dimers additionally contain non-kit ligand amino acid sequences that will

naturally form dimers, such as with Ig fusion proteins; or when the translation product is a single chain polypeptide comprising two monomers of kit ligand amino acid sequences fused to one another through a peptide linker.

Any well known crosslinking protocol may be employed to carry out the crosslinking means. These include chemical crosslinking, *in vitro* formation of disulfide bridges, the use of known bifunctional crosslinking agents, etc. Many of these techniques are described in more detail in the examples of this application. Preferably, the crosslinking means comprise denaturing the expression product under conditions which cause disruption of intrachain disulfide or other naturally occurring bonds, followed by refolding the expression product under conditions which promote formation of interchain covalent bonds. Preferred denaturing conditions include solubilization in urea, preferably 4M - 12M, most preferably 6M; or guanidine, preferably 2M, in the presence of small amounts (<1 mM) glutathione (both reduced and oxidized forms, preferably in a 4:1 ratio). Preferred refolding conditions employ dialysis against a neutral buffer, such as Tris or phosphate buffered saline (PBS).

The formation of KL-CD through expression in mammalian and other eukaryotic species is demonstrated in Example 1. The protein can also be expressed in mammalian, yeast or insect cells, then purified and subsequently denatured and refolded to facilitate intermolecular disulfide bond formation. In some cases it may be desirable to remove sugars using endoglycosidases and other enzymes to cleave sugars that interfere with intra- and/or interchain disulfide formation.

KL-CD can be expressed in prokaryotic as well as eukaryotic expression systems. The following are examples of expression vectors which may be used in procaryotic systems:

The pPL expression series use the strong PL promoter of lambda phage, and can be expressed in a number of procaryotic expression systems (Reed, Cell, 25, 713-719 (1981), Simatake and Rosenberg, Nature, 292, 128-132 (1981), Mott, et al., Proc. Natl. Acad. Sci. USA, 82, 88-92 (1985)).

The pOX expression series, which uses the oxygen-dependent promoter of *Vireoscilla* hemoglobin gene, is expressed in *E. coli* (Khosla, et al., BioTechnology 8, 554-558 (1990)).

pKK223-3 uses a hybrid promoter derived from the fusion between the promoters of the tryptophan and lactose operons of *E. coli* (Brosius and Holy, Proc. Natl. Acad. Sci. USA 81 6929-6933 (1984)).

The following are examples of expression vectors which may be used for expression in a eukaryotic expression system:

pMSG uses the promoter from the mouse mammary tumor virus long terminal repeat (MMTV). Suitable host cells for pMSG are Chinese hamster ovary cell, Hela cell and mouse Lkt negative cells (Lee, F., et al., Nature 294, 28-232 (1981)).

pSVL uses the SV40 late promoter. Suitable host cells are COS cells for high level transient expression (Sprague, et al., J. Virol. 45, 773-781 (1983); Gempleton and Eckhart, Mol. Cell. Biol. 4, 817-821 (1984)).

pRSV uses Rous Sarcoma Virus promoter. Suitable host cells are mouse fibroblast cells, lymphoblastoid cells and COS cells (Gorman, et al. Science 221, 551-553 (1983)).

pBPV is a DNA viral vector derived from bovine papilloma virus. It is stably expressed in mouse mammary tumor cells, C127 (Zin, et al., Cell 34, 865-879 (1983); Saraver, et al., Mol. Cell. Biol. 1, 486-496 (1981)); Saraver, et al., Proc. Natl. Acad. Sci., USA 79, 7147-7151 (1982); Law, et al., Mol. Cell. Biol. 3, 2110-2115 (1983)).

Baculovirus expression vectors are stably expressed in insect cells such as Sf9 (Luckow and Summers, BioTechnology, 6, 47-55 (1988); Miller, L.K., Ann. Rev. Microbiology 42, 177-199 (1988)).

Methods for making transgenic animals are well known. DNA encoding the KL can be introduced into the cells in culture using transfection or into embryos for production of transgenic animals expressing the KLs. As known in the art, transfection can be accomplished by electroporation, calcium phosphate precipitation, a lipofectin-based procedure, or microinjection or through use of a "gene gun". In each case, cDNA encoding the KL is subcloned into a plasmid-based vector which encodes elements for efficient expression in the genetically engineered cell. The plasmid-based vector preferably contains a marker such as the neomycin gene for selection of stable transfectants with the cytotoxic aminoglycoside G418 in eukaryotic cells and an ampicillin gene for plasmid selection in bacteria. In the preferred embodiment, the KL is expressed in soluble form; in the most preferred embodiment, the KL is expressed using a tissue specific protein such as the casein promoter, to avoid potential side effects and to increase recoverable yields.

Infection, which for endothelial cells is preferred, is accomplished by incorporating the genetic sequence for the KL into a retroviral vector. Various procedures are known in the art for such incorporation. One such procedure which has been widely used in the art employs a defective murine retrovirus, Psi-2 cells for packaging the retrovirus, and the amphotropic packaging cell line Psi-AM to prepare infectious amphotropic virus for use in infecting the target donor cells, as described by Kohn et al., 1987 "Retroviral-mediated gene transfer into mammalian cells" Blood Cells 13:285-298. Alternatively, rather than a defective Moloney murine retrovirus, a retrovirus of the self-inactivating and double-copy type can be used, such as that described by Hantzopoulos et al.,

1989 "Improved gene expression upon transfer of the adenosine deaminase minigene outside the transcriptional unit of a retroviral vector" Proc. Natl. Acad. Sci. USA 86:3519-3523.

A variety of methods are known to those skilled in the art for making transgenic animals expressing a KL protein. Examples of particularly useful animals include mice, rats, rabbits, pigs, sheep, and cattle, all of which have been made transgenic using standard techniques. The most well known method for making a transgenic animal is by superovulation of a donor female, surgical removal of the egg and injection of the genetic material in the pronuclei of the embryo, as taught by U.S. Patent No. 4,873,191 to Wagner, the teachings of which are incorporated herein. Another commonly used technique involves the genetic manipulation of embryonic stem cells (ES cells). ES cells are grown as described, for example, in Robertson, E.J. "Embryo-derived stem cell lines" in: Teratocarcinomas and embryonic stem cells: A practical approach. E.J. Robertson, ed. 71-112 (Oxford-Washington, D.C.: IRL Press, 1987). Genetic material is introduced into the embryonic stem cells, for example, by electroporation according to the method of McMahon, A.P., and Bradley, A. Cell 62, 1073-1085 (1991). Colonies are picked from day 6 to day 9 of selection into 96 or 24 well dishes (Costar) and expanded and used to isolate DNA for Southern blot analysis. Chimeric mice are generated as described in Bradley, "Production and analysis of chimaeric mice" in Teratocarcinomas and embryonic stem cells: A practical approach E.J. Robertson, ed. pp. 113-151 (Oxford, Washington, D.C. IRL Press 1987), the teachings of which are incorporated herein. Genetic material is injected into blastocysts. From those implanted females that become pregnant, chimeras are selected from the offspring and bred to produce germline chimeras for use as donor animals.

According to a preferred embodiment, the invention provides recombinant DNA molecules characterized by a nucleic acid sequence encoding a fusion protein comprising kit ligand amino acid sequences fused to non-kit ligand amino acid sequences which forms a kit ligand dimer of this invention *in vivo*.

b. Properties of KL-CD

In Example 1, a truncated form of murine KL encoding amino acids 1 to 164 (amino acids 1-164 of SEQ ID NO:3) plus an additional N-terminal methionine required for synthesis in *E. coli* was expressed. This form corresponds to the natural soluble form of murine KL-1. In this method, KL is synthesized and accumulates within the bacteria in an insoluble form. KL-CD is obtained by solubilization of the protein with denaturant (urea), and refolding into biologically active protein by removal of the denaturant (by dialysis into buffer such as Tris™ HCl, pH 8 - 9). During the refolding, both intrachain and interchain disulfide bonds are formed, resulting in two types of KL which can promote cell proliferation, KL-NC and KL-CD. In addition, a biologically inactive KL-CD is

formed. The three forms of KL can be separated from one another and from contaminating *E. coli* proteins using a high resolution chromatography method such as C18 reverse-phase HPLC.

Expression of KL-CD, derived from KL-cDNAs with three cysteines or with greater than four cysteines in eukaryotic cells can be facilitated by expression of the full length KL cDNA (KL-1, Huang, et al., 1992) with the appropriate Cysteine mutation such that KL dimers will associate first in the membrane and then be released via cleavage at the native proteolytic cleavage site.

Murine KL-NC described in Example 1 presumably contains the same disulfide bonds found in rat and human KL (4-89, 43-138). Peptide mapping studies involving proteolytic digestion and HPLC purification of the resulting peptides, described in Example 8, indeed revealed two different peaks containing disulfide-linked peptides. As described in detail in Example 8, the active KL-CD molecule has the same peptide map as KL-NC under both reduced and non-reduced conditions, indicating that active KL-CD and KL-NC contain the same cysteine pairings in the disulfide bonds. Thus, in the active form of KL-CD, at least one of the intramolecular disulfide bonds normally found in KL-NC has been replaced with a disulfide bond involving the same cysteine residues, but paired intermolecularly instead of intramolecularly. The inactive KL-CD molecule has a peptide map that is different than both KL-NC and active KL-CD when analyzed under non-reduced conditions. This inactive form of KL-CD therefore contains different combinations of cysteines involved in its disulfide bonds, resulting in a molecule with little biological activity

c. Covalent dimers of KL-related proteins

The approaches outlined above for the formation of a covalent dimer of KL may also be applied to the formation of covalent dimers of other non-disulfide linked multimeric proteins. In particular, covalent dimers of FL are expected to possess increased activity because of its non-covalent nature and overall structural similarity with KL. The amino acid sequence of the murine FLT-3 ligand is shown as SEQ ID NO.:6, as reported by Lyman, et al., (1993) Cell 1157-1167. The full length cDNA can be expressed in eukaryotic cells with vectors specified and soluble protein recovered after proteolytic cleavage via the endogenous protease in CV-1 cells. Alternatively, a soluble form of FLT-3/FLK-2 ligand can be isolated from eukaryotic or prokaryotic cells by expression of a fragment of the cDNA, for example, from amino acid one to 135 or one to 163. Cysteines at positions 119, 124, and 130 can also be replaced by other amino acids, preferably serine. Other modifications such as additional cysteines, in the same region as specified for KL as well as fusion proteins can also be used to produce disulfide linked FLT-3/FLK-2 ligands.

Formation of covalent dimers of the FLT-3/FLK-2 ligand are expected to have desirable biological properties similar to that of KL, including increased potency in stimulating proliferation of bone marrow subpopulations, and increased stability. Biologically active, disulfide-linked covalent dimers of FLT-3/FLK-2 ligand may be obtained more easily with the human form which contains six

cysteine residues, rather than with the mouse form, which contains nine cysteines. As with KL, covalent dimers of FLT-3/FLK-2 ligand may be formed by denaturation and refolding, through the addition of cysteines in the region of amino acid 31, via fusion proteins, or by chemical crosslinking means.

d. Biological Activities and Applications of KL-CD

Native KL has multiple biological activities, affecting the growth and differentiation of a variety of hematopoietic cells, as well as the activation of mast cells. While the mast cell activating property of native KL limits its utility as a therapeutic, KL-CD has properties which make it useful for applications that were originally intended for native KL. As described in Example 3, murine KL-CD is at least ten-fold more potent than murine KL-NC as well as human KL-NC in stimulating the proliferation of two different human cell lines, and ten-fold more potent than murine KL-NC in stimulating the proliferation of murine mast cells. However, KL-CD is only equipotent to that of murine KL-NC in priming mast cells for IgE-dependent degranulation. The P:A ratio for KL-CD is thus ten fold more favorable than the P:A ratio of KL-NC. This differential increase in growth stimulation in contrast to mast cell priming-activation of KL-CD is of utmost importance since the KL-induced anaphylaxis is presumably due to its action on mast cells.

The selectivity of KL-CD for promoting cell proliferation but not mast cell degranulation, may make the disulfide-linked form particularly useful as a therapeutic since dosages may be set which promote a desired proliferation event but which avoid mast cell degranulation-induced anaphylaxis. For example, since KL-CD is ten-fold more potent than KL-NC in promoting cell proliferation, a dose of 10 µg/kg/day of KL-CD should be as effective as a 100 µg/kg/day dose of KL-NC, a dose which stimulated significant hematopoietic recovery. Since KL-CD is equipotent to KL-NC in promoting mast cell degranulation, a dose of 10 µg/kg/day of KL-CD is below the dose of 25 µg/kg/day which resulted in mast cell-related side effects in some patients, and well below the dose of KL-NC of 100 µg/kg/day which resulted in serious mast cell-related effects in many patients.

KL-CD can be used to stimulate hematopoietic recovery following chemo/radiotherapy or bone marrow (hematopoietic cell) transplantation, as previously described in the literature, and reviewed in the Background of the Invention. This may be accomplished with KL used as a single agent or in combination with other cytokines, such as G-CSF or GM-CSF for neutrophil recovery, or IL-6 or other factors that promote platelet recovery. KL-CD may also be more effective in treating certain anemias such as those associated with Diamond Blackfan Syndrome, those induced by chemo or radiotherapy or viral infections, or aplastic anemia. The dimer will also be useful in the mobilization of stem cells from the bone marrow to the peripheral blood alone and or in combination with other cytokines, such as G-CSF, or chemotherapy. Since KL-CD would be used at the same dose as KL-NC, which is limited by its toxicity, KL-CD should be significantly more effective than

KL-NC in the aforementioned applications, due to its enhanced potency in promoting cell proliferation.

While short-term exposures of mast cells in culture to KL results in mast cell priming, i.e. in enhanced IgE-dependent mast cell degranulation, prolonged exposure of these cells to KL results in a desensitization of the priming effect. This suggests that patients could be desensitized to the mast cell activating effects of KL by treatment with a level of KL-CD below the toxicity level. A subsequent treatment of high level KL-CD or KL-NC might then provide enhanced hematopoietic recovery without causing mast cell associated toxicity. The level of hematopoietic recovery might be greater than that observed for KL-CD used at a level below the toxicity level. In summary, KL-CD or KL-NC should be useful in a desensitization protocol to establish a higher toxicity level for KL.

KL-CD should also have utility for *ex vivo* applications. Although the differential proliferative/mast cell activating property of KL-CD is less important for *ex vivo* uses, its increased biological activity make it useful in the culture of hematopoietic cells. KL is effective by itself or preferably in combination with other cytokines, such as IL-1, IL-3, IL-6, IL-11, G-CSF, GM-CSF, LIF, FLT-3/FLK-2 ligand and combinations thereof, for the *ex vivo* expansion of stem cells and progenitors for transplantation. The ability of KL-CD to stimulate the proliferation of immature stem/progenitor cells makes KL particularly useful in protocols involving the transduction of genes into hematopoietic cells for gene therapy. KL-CD could be used at lower dosages relative to KL-NC for these *ex vivo* applications. KL-CD might also result in qualitative differences in hematopoietic cell expansion compared to KL-NC, perhaps resulting in the selective expansion of a certain type of progenitor cell.

KL-CD is also significantly more stable than KL-NC. The increased stability of KL-CD is particularly apparent at low concentrations, between 1 and 100 ng/ml, when incubated at 37°C for several days to weeks. At these concentrations, significant loss in activity is observed for the recombinant KL-NC.

The greater stability of KL-CD relative to KL-NC may enhance the utility of KL-CD in *ex vivo* applications. KL-NC exhibits properties *in vitro* which suggests an inherent instability of the molecule, perhaps due to the dissociation of the dimer into monomers at lower protein concentrations, or to internalization and degradation of the molecule by the responding cells. This is illustrated by repeated daily feeding of mast cell cultures with KL which gives significantly better growth than two to three times a week feeding. It may be possible to use the covalently-linked KL dimer to overcome this apparent instability, and allow one to use a significantly lower concentration of soluble KL-CD to support long term cell cultures.

In summary, KL-CD can be utilized as an additive to cell culture media as extrapolated from the published data relating to KL, or in combination with a pharmaceutically acceptable carrier

for administration to a patient. Exemplary pharmaceutical carriers include diluents such as saline and phosphate buffered saline, additives such as preservatives, detergents, solubilizing agents, anti-oxidants, pH buffers, and salts, as well as alternative carrier forms such as polymers, liposomes, micelles, and vesicles. These are administered to a patient in an amount effective to produce an improvement in a particular condition, for example, to increase platelet numbers. Treatment may be alone or in combination with other compounds demonstrated to have hematopoietic activity, including erythropoietin, G-CSF, GM-CSF, interleukins 1-11, IGF-I, FLT-3/FLK-2 ligand or LIF.

In addition to the above-described covalent dimers, stabilized dimers with increased biological activity can also be produced through non-covalent means by the fusion with domains that readily form stable hetero- or homomeric multimers. An example would be to use the so called "Leucine zipper" domain which will self associate with another protein that contains a Leucine zipper domain. Such dimers are also within the scope of this invention.

Example 1: Purification and Characterization of Covalent Dimer-Kit Ligand (KL-CD) from Native KL Sequence.

A truncated mouse KL cDNA encoding amino acids 1 to 164 (amino acids 1-164 of SEQ ID NO:3) plus an N-terminal methionine was subcloned from the full length cDNA. The endpoint of this truncated cDNA was chosen based on the site of proteolytic cleavage in the native transmembrane form of the molecule which gives rise to the soluble form (Huang et al., 1992). The truncated KL cDNA was cloned into an expression vector, placing it under control of the phage lambda early gene promotor P_L (Lambda II, Hendrix, Roberts, Stahl, Weisberg, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1983)). This promoter is regulated in a temperature-sensitive manner by a mutant phage lambda CI gene which is also present on the expression vector. The truncated cDNA was expressed in *E. coli* strain DH5-a (BRL-GIBCO). As is typical for high level expression in *E. coli*, the truncated KL accumulated in an insoluble form in inclusion bodies in the bacteria.

A two liter culture of *E. coli* expressing KL was harvested, the cells were lysed by sonication, and the inclusion bodies containing insoluble KL were isolated by centrifugation at 10,000 x g. The inclusion bodies were washed by resuspension in 20 mM Tris HCl, pH 8.5, 200 mM NaCl 1 mM EDTA and re-centrifuged. The inclusion bodies were solubilized by incubation in 6 M urea at 4°C for 1 h, followed by centrifugation to remove insoluble material. After solubilization of inclusion bodies containing mKL, the protein was dialyzed against 20 mM Tris pH 8.0 at 4°C for 48 to 72 h. Insoluble material was removed by centrifugation at 10,000 x g, and the protein was applied to a Vydak™ C18 1 x 25 cm HPLC column that had been equilibrated with 0.1 M ammonium acetate pH

6.0 and 25% n-propanol. The column was washed with equilibration buffer, and then eluted with a linear gradient from 30-50% n-propanol, 0.1 M ammonium acetate pH 6.0.

mKL bioactivity, as measured by the ability to promote proliferation of the cell line MO7e described in Example 2, elutes in two peaks; non-covalently linked mKL (KL-NC) elutes at approximately 38% n-propanol, KL-CD elutes at approximately 45% n-propanol, as shown in Figure 2A. A third peak containing a different form of KL-CD with very low activity elutes after the biologically active KL-CD peak, as also shown in Figure 2A.

The KL-NC and KL-CD peaks were purified to homogeneity by re-application to the C18 column, and elution with narrower gradients. KL-NC was purified using a gradient from 32-45% n-propanol. The active and inactive KL-CD forms were purified using a 2 h gradient of 35-45% n-propanol. After the second C18 column, the NC and CD forms were in a highly purified state, and contained low levels of *E. coli*-derived endotoxin (less than 1 E.U. per mg protein as assayed by the BioWhittaker Inc. Amebocyte Lysate Assay). Prepared through these means, approximately 15% of the mKL refolds into active KL-CD, 15% into inactive KL-CD, and 70% into KL-NC.

The difference between KL-NC and the two KL-CD forms can be seen not only by their different retention times on the C18 column, but by SDS-PAGE under reducing/non-reducing conditions, as shown in Figure 2B. Under reduced conditions, KL-NC as well as the two forms of KL-CD migrate with an apparent molecular weight of about 18 kDa. Under non-reduced conditions, KL-NC migrates with an apparent molecular weight of about 18 kDa, while the two different forms of KL-CD migrate with an apparent molecular weight of 36 kDa. As assessed by SDS-PAGE under non-reducing conditions, the active form of KL-CD has a slightly greater apparent molecular weight than the inactive form of KL-CD. The higher apparent molecular weight of KL-CD as compared with KL-NC under non-reducing conditions is indicative of the covalent linkage of two KL monomers via at least one disulfide bond.

The nature of KL-CD and KL-NC has been confirmed by Laser Desorption/Time of Flight Mass Spectrometry. By this method, KL-NC has a mass of 18,440 daltons. Both active and inactive KL-CD had a mass of 36,860 daltons; these forms apparently differ only in their disulfide bonds, with inactive KL-CD containing a disulfide bond arrangement which greatly diminishes activity.

Example 2: Formation of KL-CD from KL-NC via disulfide rearrangement.

KL-CD can also be derived from pure KL-NC through a non-enzymatic reaction involving the re-arrangement of disulfide bonds. The reaction consists of pure, correctly folded KL-NC at 1 mg/ml, 50 mM Tris pH 9.0, 2 M guanidine-HCl (added to partially unfold the KL-NC), and reduced and oxidized forms of glutathione (500 µM and 125 µM final concentration, respectively). The reaction mixture was incubated for 20 h at 22°C, and then dialyzed against 0.1 M ammonium

acetate at 4°C to remove the guanidine to allow folding and to stop disulfide exchange. The rearrangement reaction was monitored by SDS-PAGE under non-reducing conditions.

Proteins with molecular weights of the active and inactive forms of KL-CD were formed via rearrangement of KL-NC disulfides. This rearrangement required the presence of 2 M guanidine-HCl. Additionally, several other KL species were formed, which might be inactive forms of KL-CD. The mixture of proteins resulting from disulfide rearrangement of KL-NC was purified by C18 reverse phase chromatography as in Example 1.

Figure 3A is a photograph of SDS-PAGE of KL-CD and KL-NC refolded from KL-NC in the presence or absence of glutathione. Figure 3B is a chromatogram of a C18 reverse phase HPLC separation of refolded material as shown in Figure 3A. Figure 3C is a graph of KL bioactivity of fractions from the chromatogram shown in Figure 3b.

Two peaks of biologically active KL were identified; the second peak consisted of KL-CD which migrates with the same apparent molecular weight of KL-CD purified as in Example 1 in SDS-PAGE under reducing conditions. This shows that KL-CD with increased biological activity can be formed from KL-NC through disulfide rearrangement.

Disulfide rearrangement conditions can be established which maximize KL-CD formation. It may be preferable however to purify recombinant KL in a completely unfolded state by C18 reverse-phase HPLC, and then fold the protein into CD- and NC- KL forms using the disulfide-rearrangement conditions described above.

Example 3: Biological activity of KL-CD in *in vitro* biological assays.

a. Cell proliferation.

KL supports the proliferation of a variety of growth factor dependent cell lines. Murine KL is equally potent on both human and murine cells, while human KL is active on human cells but shows minimal activity on murine cells. The human megakaryocytic cell line M07e, which is maintained in the presence of GM-CSF, is used to assess human and murine KL. Murine bone marrow-derived mast cells (BMMC), which are established and then maintained for up to three months in the presence of IL-3 (Yung, Y.P., et al. (1982) J. Immunol., 129, 1256-1261), are also used to assess the activity of murine KL (Nocka, K., et al. (1990).

The cells are washed and resuspended in media lacking their maintenance growth factor and plated into 96 well plates. Column fractions of KL samples are added and serially diluted and the cells are incubated at 37°C for 24 h. The cells are then pulsed with 2.5 µCi/ml of [3]H-Thymidine for 6-12 hr, harvested onto glass fiber filters, and the amount of ^{3}H-thymidine on the filter is determined on a Packard TopCount™ scintillation counter. The data is analyzed by plotting the CPM ^{3}H incorporated into DNA versus the concentration of KL.

Although much greater amounts of KL-NC were present after disulfide rearrangement, C18 fractions containing KL-NC or KL-CD had comparable activity in promoting cell proliferation, as shown by Figure 4.

b. Mast Cell Priming Activity

Primary cultures of murine mast cells derived from bone marrow and cultured in IL-3 (BMMC) can be utilized not only for proliferation assays as described above, but may also be used as a quantitative and sensitive measure of the priming or activation potential of cytokines. With human mast cells, KL is the most potent cytokine identified to date with significant mast cell priming activity *in vitro* (Bischoff and Dahinden (1992) J. Exp. Med., 175, 237-244). Murine BMMC sensitized with IgE immunoreactive with trinitrophenol (TNP) (ascites from IGELa2, ATCC # TIB 142) can be primed by KL such that when stimulated with specific antigen (TNP-BSA), exhibit a significant increase in the release of mediators compared to unprimed cells. When BMMC derived from the C57/Bl6 X DBA2 F1 (BDF1) strain of mice are activated at low cell density (1 X 10^5 cells/ml) in physiological buffer, low levels of proinflammatory mediators and secretory granule enzymes, typically 10-25% of their granule hexoseaminidase, are released upon stimulation with IgE and antigen alone. Following a short priming period with KL (0 to 10 minutes), maximal granule enzyme release in the range of 40 to 60% enzyme release is observed. Figure 5 is a graph of mast cell activation by purified KL-NC and KL-CD as a function of concentration. The mast cell priming activity of native KL has an ED_{50} of 0.5 to 1 ng/ml in this assay.

Example 4: Desensitization of mast cells to a KL response.

Human lung and murine bone marrow derived mast cells respond to the exposure of various priming agents with very rapid kinetics, as described by Bischoff and Dahinden (1992). The priming assay as specified in Example 3 is typically carried out with a priming period of 5 to 10 minutes followed by the addition of antigen for a further period of 10 minutes. As shown below, if the antigen is withheld for 30 minutes or longer, the priming affect of KL is lost and the level of degranulation is similar to that seen with antigen alone. Furthermore, BMMCs can no longer respond to a second treatment with KL when they have already been desensitized. Once the effect of Kl is lost, mast cells cannot respond to a second dose of KL within a one to two hour period. This desensitization could be used therefore to minimize a response to a subsequent therapeutic dose of KL.

a. Kinetics of Priming with KL

BMMCs which had been previously sensitized with IgE (anti-TNP) were incubated with control diluent or KL for various periods of time (0, 2, 5, 7, 10, 20, 30, 40, 50, and 60 minutes) and then activated with antigen (TNP-BSA). Percent release of hexoseaminidase was determined ten minutes after the addition of antigen.

Exposure of cells to KL for up to ten minutes prior to antigen resulted in maximal activation, as shown by Figure 6. With exposure of cells to KL for 20 minutes and longer, no significant release was observed above that seen with antigen alone.

b. Desensitization of mast cells with KL

Sensitized BMMCs were incubated in the presence or absence of KL for 45 minutes (1st phase). Following this incubation period, cells were washed and BMMCs were primed with either control diluent, or KL for 10 minutes (2nd phase). Cells were then activated by the addition of antigen for a further 10 minutes. Cells that had been cultured in the control medium for the 45 minute period responded to antigen and exhibited a significant enhancement when treated with KL as the second agent and then antigen. However, cells that had been pretreated with KL were only activated to the level seen with antigen alone. Secondary stimulation with KL did not lead to enhanced degranulation, as shown by Figure 7.

Example 5: Hematopoietic progenitor colony assays.

The ability of murine KL-CD vs. KL-NC to support the proliferation and differentiation of relatively mature as well as immature myeloid progenitor cells was evaluated by the standard Colony Forming Unit - Granulocyte/Macrophage (CFU-GM) assay for mature progenitors and the High Proliferative Potential - Colony Forming Cell (HPP-CFC) assay for immature progenitor cells. KL has been previously shown to be active in both assays with murine bone marrow cells.

a. CFU-GM assay

Murine bone marrow cells were isolated from C57Bl/6 X DBA2 F1 (BDF1) female mice and plated at a concentration of 75,000 cells/ml in standard semisolid media containing 0.3% agar. Seven days after incubation of cultures at 37°C, colonies were scored under an inverted microscope.

Murine KL-CD and KL-NC was tested starting at 50 ng/ml (1250 pM) and titrated in 2 fold serial dilutions to 0.09 ng/ml (2.44 pM). Both forms of KL stimulated colony growth from murine bone marrow cells. Similar to what had been observed for proliferation assays, lower concentrations of KL-CD were required to stimulate colony formation (Figure 8A and 8B). Doses required to stimulate 50% maximal colony formation were approximately 43 pM for KL-CD versus 347 pM KL-NC. The total number of colonies observed at a maximal dose of KL-CD was also significantly higher than what was observed for KL-NC at the maximum dose tested (50 ng/ml). This increased number of colonies suggests that KL-CD is able to affect the growth of an additional population of progenitor cells whose growth is not supported by KL-NC.

Example 6: Murine in vivo mast cell activation: Cutaneous anaphylaxis.

KL when injected intadermally into the ear of a mouse leads to mast cell activation without any requirement for a second activating signal, i.e., IgE. Mast cell activation was quantitated by measuring the edema that forms with 5 - 30 minutes following injection of KL. This edema was visualized by the accumulation of the dye Evan's Blue in the ear, which had injected i.v. 60 minutes prior to KL.

KL was injected into the right ear of CD-1 mice in a volume of 25 µl and PBS was injected into the left ear to serve as a control. Sixty minutes later the animals were sacrificed and photographed. KL-NC was tested at 60, 30, 10, 3, 1, 0.1 µg/kg. KL-CD was tested at 10, 3, 1, 0.3, 0.1 µg/kg. The results are presented in Table 1 below.

Dose (µg/kg/ear)	KL-NC	KL-CD
60	++++	ND
30	++++	ND
10	++++	++++
3	++++	+++
1	++++	+++
0.3	ND	++
0.1	+	+

ND = not determined

Maximal activation was observed with KL-NC at 30, 10, 3 µg/kg. KL-CD also led to maximal activation at 10 and 3 µg/kg. At lower doses of KL-NC and KL-CD the affect on mast cell activation titrated similarly with only minimal edema formation at the lowest concentration tested for both KL-CD and KL-NC (0.1 µg/kg). These results demonstrated that there was no increase in the ability of KL-CD to trigger mast cell activation in vivo as compared to KL-NC.

Example 7: Construction, expression and biological activity of KL-Ig fusion protein.

Human and murine KL cDNA's were fused to immunoglobulin heavy chain gene fragments in order to generate disulfide linked dimeric KL fusion proteins. A cDNA (SEQ ID NO:7) encoding a fusion protein (SEQ ID NO:8) consisting of the murine KL signal sequence, amino acids 1-165 of murine KL and amino acids 237-469 of the murine immunoglobulin heavy chain (gamma 2a isotype) was created by PCR cloning. As a result of the cloning strategy, amino acid 237 of the Ig

heavy chain, normally a glutamic acid residue, was changed to aspartic acid. When expressed in mammalian cells, the signal sequence is processed to produce the mature fusion protein (amino acids 1-400 of SEQ ID NO:8)

A human KL-Ig fusion protein construct was created by PCR amplification of an hKL fragment containing the KL signal peptide sequence and amino acids 1-165 from a human KL cDNA clone. The "sense" strand PCR primer was SEQ ID NO:9: 5'GACTCGAGCCACCAATGAAGAAGACACAAACTTGG3', which encodes an XhoI restriction enzyme site. The "antisense" strand PCR primer was SEQ ID NO:10: 5'TCAGGGATCCGCTGCAACAGGGGGTAACATAAA3', which encodes a BamHI site. The PCR product was cloned into the PCR cloning vector, PCRII vector (Invitrogen). This plasmid was digested with XhoI and BamHI to generate the hKL fragment containing the signal sequence and AA1-165. This fragment was cloned into the XhoI/BamHI restriction sites of the Ig fusion vector CD5-IgG1 (Aruffo et al., Cell, 61, pp. 1303-1313 (1990)). The DNA sequence encoding this fusion protein is shown in SEQ ID NO: 11. The unprocessed expression product is shown in SEQ ID NO: 12. When expressed and processed in COS cells, a fusion protein is produced containing amino acids 1-165 of human KL fused to 234 amino acids of the human IgG1 heavy chain (amino acids 1-399 of SEQ ID NO: 12).

DNA sequencing of the human KL-Ig construct revealed a mutation in the codon for amino acid #38 (GTT→ATT) which resulted in a valine→isoleucine mutation. Additionally, silent mutations were found in the following codons: AA#24 AAA→AAG, AA#83 GTC→GTG, AA#90 GTC→GTG, AA#165 GCC→GCG. To correct the Val→Ile mutation at AA#38 back to wild type, site directed mutagenesis on the hKL cDNA was performed. A 151 bp AatII-SspI DNA fragment from human kit ligand cDNA (nucleotides 45-195 of SEQ ID NO:1) encompassing the corrected sequence was isolated and "swapped" with the corresponding DNA fragment containing the mutation from the hKL/PCRII plasmid. The XhoI/BamHI fragment containing the corrected sequence was then cloned into the Ig fusion vector. The corrected hKL-Ig construct (SEQ ID NO: 13) was transiently expressed in COS cells, and the KL-Ig protein (amino acids 1-399 of SEQ ID NO: 14) isolated by chromatography on Protein A-Sepharose. *In vitro* and *in vivo* activity of the mutant and corrected hKL-Ig proteins were equivalent.

a. Purification of KL-Ig fusion proteins

These KL-Ig proteins were expressed transiently in Cos-7 cells which were transfected by human or mouse KL-Ig constructs in the CDM8 vector (B. Seed, Nature, 329, pp. 840-42 (1987)) by electroporation. Serum-free supernatants were collected from the Cos-7 cells daily for up to ten days after transfection and tested for biological activity. Active collections were pooled and KL-Ig was purified on Protein A sepharose. Protein was quantitated by the BCA protein assay reagent

(Pierce, Rockford, IL) as well as by an anti-human or anti-murine KL ELISA. Active protein was detected for both the human and murine KL-Ig fusion proteins.

b. Identification of KL-Ig Fusion Proteins by Metabolic Labelling and Immunoprecipitation

The expression of KL-muIg and KL-huIg was assessed using supernatants collected from COS-7 cells transiently transfected with plasmid DNA. The transfected cells were pre-cultured (30 minutes, 37°C) with labeling medium (Earl's Salts, Vitamins, essential and non-essential amino acids, 2% dialyzed fetal bovine serum) deficient in methionine and cysteine, followed by the addition of [^{35}S]-methionine, [^{35}S]-cysteine (Expre^{35}S-^{35}S Labeling Mix, New England Nuclear, Wilmington, DE) to 100 µCi/ml for four hours at 37°C in a 5% CO_2, humidified atmosphere. The conditioned medium was then collected and allowed to react either with anti-mouse IgG sepharose or Protein G sepharose for 12 hours at 4°C on a rotating mixer. The sepharose beads were subsequently washed (0.1% NP-40, 150 mM NaCl, 20 mM Tris, pH 7.4) three times prior to the addition of reducing SDS-PAGE sample buffer. The samples were heated to 100°C for five minutes and the supernatants were analyzed by SDS-polyacrylamide gel electrophoresis (4-12% gradient), followed by autoradiography. This analysis revealed a 58 KDa band for the KL-muIg construct and a 53 KDa/58 KDa doublet for the KL-huIg fusion protein. Analysis of the murine Ig-fusion protein under non-reduced conditions revealed a band at 130-140 KDa. This band probably represents a disulfide linked dimer form of the 58 Kda monomeric band.

c. Biochemical Characterization of the KL-Ig Fusion Proteins

When analyzed by SDS-PAGE under reduced conditions, purified mouse and human KL-Ig fusion protein migrates as a "cluster" of three distinct bands of approximately 55-60 kDa. Also present are several bands of between 30-40 kDa that are likely proteolytic fragments. Under non-reduced conditions, two prominent bands of approximately 120 kDa are observed, consistent with the idea that these proteins consist of two disulfide-bonded 60 kDa monomers. There are also lesser amounts of 60-80 kDa bands (disulfide-linked proteolytic fragments), as well as a 200 kDa band that is likely an aggregate of the full length dimer.

The glycosylation state of the mouse and human KL-Ig fusions was investigated through digestion with the endoglycosidases N-glycosidase F, O-glycanase and the exoglycosidase sialidase, followed by SDS-PAGE. Digestion with all three enzymes reduces the 55-60 kDa cluster (under reduced conditions) to a single band of 50 kDa. Further, these experiments show that a) N-linked glycosylations are present, and b) the heterogeneity in molecular weight of the 55-60 kDa cluster is due to either heterogeneity in the amount of either O-linked glycosylation or the amount of sialic acid addition.

As assessed by HPLC-gel filtration and analysis of eluted fractions, mKL-Ig and hKL-Ig form biologically active (measured in the MO7e proliferation assay) aggregates in solution. Approximately 50% of the hKL-Ig migrates with an apparent molecular weight of about 200 kDa, with the remainder of the protein eluting as distinct peaks with molecular weights in the range of 400 to 2,000 kDa. About 25% of mKL-Ig migrates as a 200 kDa species, the remainder as 400 to 2,000 kDa.

d. Biological Activities of KL-Ig fusion proteins

The specific activity of the KL-Ig fusions were compared with mKL-NC and mKL-CD in a proliferation assay of the human factor dependent cell line, MO7e (Figure 9). The specific activities of both murine and human KL-Ig molecules is comparable to the level of activity of KL-CD (0.5-1.0 ng/ml) and is significantly higher than that of KL-NC (6-15 ng/ml). On a molar basis, since both fusion proteins are much larger than mKL-NC and mKL-CD, their potency is very similar to that which is observed for KL-CD.

Murine KL-Ig was also tested for its ability to prime BMMC which were then triggered with IgE + antigen for degranulation. A titration of KL-Ig, KL-NC and KL-CD demonstrated that KL-Ig was slightly less active than KL-CD and KL-NC when compared on a weight basis (ng/ml) (Figure 10). However, on a molar basis mKL-Ig is equipotent with KL-NC.

Example 8: Disulfide Bonds of murine KL-CD

Peptide mapping was performed to determine the disulfide bond pairs in the active and inactive forms of KL-CD, as well as KL-NC. Protein was enzymatically digested with endoproteinase Asp-N, which cleaves peptide bonds N-terminally at aspartic acid residues. After digestion, some of the sample was incubated with 15 mM dithiothreitol (DTT) to reduce the disulfide bonds, and then treated with 20 mM iodoacetamide to alkylate the free sulfhydryl groups to prevent re-formation of disulfides. The peptide maps of fully reduced and non-reduced protein digest were then analyzed by reverse-phase HPLC using a C18 column and an acetonitrile/TFA gradient.

As previously reported (Langley et al., (1992) Arch. Bioch. Biophys. 295, 21-28), recombinant human and rat KL expressed in E. coli and CHO cells have intrachain disulfide bonds between cysteines at amino acids 4 and 89 and between 43 and 138. The peptide map of KL-NC reveals two peaks, labeled X and Y in Figure 11A, which are present in the non-reduced digest, but missing from the fully reduced/alkylated digest. Peaks X and Y each contain two disulfide bonded peptides. Peak X, when isolated, resolves as a single peak under non-reduced conditions, but gives rise to two new peaks, labeled X1 and X2, when reduced with DTT (Figure 11B). The first 8

amino acids of Peak X1 contain the sequence DCVLSSTL (amino acids 137-144 of SEQ ID NO:4), corresponding to amino acids 137-144 of mKL, while the first 8 amino acids of Peak X2 contains the sequence DVLPSHCW (amino acids 37-44 of SEQ ID NO:4), corresponding to amino acids 37-44.

Similarly, isolated Peak Y gives rise to a new peak labeled Y1 upon reduction; the peak labeled Y2 is likely the peptide disulfide bonded to Y1 (Figure 11B), although this peak was not isolated and sequenced. The sequence of the first 8 amino acids of peptide Y1, MKEICGNP (amino acids 1-7 of SEQ ID NO:4, plus an N-terminal methionine), corresponding to the added methionine and amino acids 1-7. This data indicates that peak X is a dipeptide linked via cysteines 43 and 138, and that Peak Y is a dipeptide linked via cysteines 4 and 89. Thus, KL-NC has the same disulfide pairs as is found in human and rat KL.

The reduced/non-reduced peptide maps of the active form of KL-CD are identical to those of KL-NC (Figures 11C and 12D) indicating that active KL-CD also has the Cys4-Cys89 and Cys43-Cys138 disulfide pairs. Additional experiments are needed to determine if active KL-CD has one or both of the disulfide pairs in the intermolecular configuration.

Peptide mapping of the inactive form of KL-CD shows that this form has the Cys43-138 disulfide pair, but lacks Peak Y (the Cys4-89 disulfide) (Figure 11E). As seen in the peptide map under non-reduced condition (Figure 11 E), this form appears to have an alternative disulfide (peak Z), and possibly a third type of disulfide (peak ZZ). Under reduced conditions, the peptide map of the inactive form is identical to that of KL-NC and active KL-CD (Figure 11 F), indicating that inactive KL-CD is made up of the full length KL protein. The covalent nature of inactive KL-CD could be due to Cys4-Cys4 and/or Cys89-Cys89 disulfides (possibly corresponding to peaks Z and ZZ in the peptide map) between two KL monomers.

Example 9: Formation and Activity of Human KL-CD

To determine if human KL could form a biologically active protein with interchain disulfide bonds, we sought to re-fold human KL-NC into KL-CD. Purified human KL containing amino acids 1-165 (SEQ ID NO:2), derived from protein expressed in *E. coli* from DNA having the sequence shown in SEQ ID NO:1 plus a 5' ATG initiation codon was incubated with 10 mM DTT at 50°C for 15 minutes to reduce the disulfide bonds. The reduced protein was incubated in refolding buffer (50 mM Tris-HCl pH 9.0, 2M guanidine-HCl, 0.5 mM reduced glutathione, 0.125 mM oxidized glutathione, 1 mg/ml KL) for 24 hours to allow disulfide bond formation, and dialyzed against 20 mM Tris-HCl pH 8.0 to allow complete re-folding.

The re-folded human KL and the starting human KL sample were then purified by C18 reverse-phase HPLC using a gradient from 25% to 70% n-propanol/ 0.1 M ammonium acetate pH

6.0 (from 15' to 105'; Figure 12, panel A), and fractions were analyzed for their ability to promote proliferation of the MO7e cell line (FIgure 12, panel B), and for protein content by SDS-PAGE (Figure 12, panel C) under non-reduced conditions. The starting human KL sample resolved as a single peak of growth stimulating activity, but the refolded sample resolved as two peaks of growth stimulating activity (Figure 12, panels B). The first peak of activity contains human KL which migrates as an 18 kDa protein under non-reducing conditions (Figure 12. panel C, lane 3; minute 46 from chromatogram), and the second peak of activity contains a 36 kDa protein which co-migrates with murine KL-CD (active) (Figure 12, panel C lane 7; minute 54 from chromatogram). Additionally, fractions lacking growth promoting activity, but containing a protein which co-migrates with inactive murine KL-CD was detected in the refolding reaction (Figure 12, panel C, lane 9; minute 60 from chromatogram).

These data show that human KL can be refolded into biologically active protein containing an interchain disulfide bond (KL-CD). Judging by the A_{280} of the first (KL-NC) and second (KL-CD) peaks of activity, the human KL-CD appears more active (approximately 10-fold) than human KL-NC in stimulating growth of MO7e. As is the case for murine KL, human KL appears to form both active and inactive forms of KL-CD.

Example 10: Mobilization of hematopoietic progenitors *in vivo* in mice

a. KL-CD

The mobilization and expansion of progenitors from the marrow to the peripheral blood and spleen is one of the pharmacological activities which can be used to determine the relative potency or activity of kit ligand *in vivo*. Mice were injected with KL-NC or KL-CD or PBS control by subcutaneous injection with 10, 30, or 100 µg of KL/kg body weight of BDF1 mice for 5 days. On day 6 the animals were sacrificed, bled by cardiac puncture and spleens removed and disaggregated. Peripheral blood or spleen cells were set up in standard CFU-GM assays and colonies quantitated after 7 days of culture. With this dosing regimen no significant effect on progenitor cell numbers was observed with KL-NC at any of the doses tested. In contrast, KL-CD did exhibit a modest level of activity at 30 µg/kg, which became significant at 100 µg/kg (Figure 13).

The same experiment was repeated using continuous infusion of KL-NC or KL-CD in order to maintain KL levels throughout the 24 hour period. Alza osmotic minipumps were filled with KL-NC or KL-CD at concentrations such that they would deliver 30 or 100 µg/kg for 6 days. Pumps were implanted subcutaneously and animals sacrificed on day 6 and spleen and peripheral blood cells set up in CFU-GM assays. A much greater effect of KL-NC and KL-CD was observed than with the once a day subcutaneous injections. As seen in Figure 14, little to no effect was observed

with KL-NC at 30 µg/kg/day; however 5- and 38-fold increases in CFU-GM were noted in the blood and spleen, respectively, at 100 µg/kg/day. The effect of KL-CD was also significantly greater with 7- and 19-fold increases seen at 30 µg/kg/day in the peripheral blood and spleen, respectively. At 100 µg/kg/day a 28-fold increase was seen in the peripheral blood and a 115-fold increase observed in the spleen. This experiment clearly demonstrates the increased activity of KL-CD over KL-NC *in vivo*.

b. KL-Ig Fusion protein

Murine KL-Ig fusion protein was also tested for *in vivo* activity. Due to the relatively large size of KL-Ig it was expected that the absorption of KL-Ig following subcutaneous administration might be limited so KL-Ig was administered by i.v. at 10, 30, 100, and 200 µg/kg day. KL-NC was given at 100 and 200 µg/kg/day as a control. Animals were sacrificed on day 6 and progenitors quantitated. As demonstrated in Figure 15, KL-Ig stimulated a significant increase in progenitors in the spleen and blood at 30, 100, and 200 µg/kg/day. KL-NC was active at 100 µg/kg, but had little activity at 200 µg/kg/day.

Example 11: Formation of KL Dimers Containing Additional Cysteines

DNAs encoding kit ligand dimers containing additional cysteines were created using standard site-directed mutagenesis techniques.

The human KL cDNA depicted in SEQ ID NO:1 was mutated to create tyrosine-to-cysteine substitution at amino acid 26 through site-directed mutagenesis using the antisense primer: 5'-GAGGGTTATCATGCAGTCTTTTGGAAG-3' (SEQ ID NO.:15). The same starting human KL cDNA was also mutated to add an additional cysteine between amino acids 26 (tyrosine) and 27 (methionine) using the antisense primer: 5'-GAGGGTTATCATGCAGTGTCTTTTGG-3' (SEQ ID NO:16).

The resulting cDNAs (SEQ ID NO:17 and SEQ ID NO:19) were cloned into an expression control under control of the P_L promoter and are expressed as described in Example 1. Inclusion bodies formed in the recombinant bacteria are isolated, denatured, refolded and the covalent dimers isolated on reverse phase HPLC, as described above. Fractions from the HPLC column are assayed for biological activity and to distinguish between active and inactive forms of covalent KL dimers. SDS-PAGE of the active fractions under both reducing and non-reducing conditions is used to distinguish intrachain disulfide bonded monomeric forms from the desired covalent dimers.

The isolated covalent KL dimers (SEQ ID NOS: 18 and 20) display greater cell proliferative activity than the monomeric forms, but no concomitant increase in mast cell activation.

Example 12: Formation of Linker Connected KL Fusion Dimers

DNA molecules which encode two molecules of human kit ligand amino acids 1-165 linked to each other via a 12 or 22 amino acid linker were constructed as follows.

An XbaI site and a KpnI were respectively added to the beginning and end of the DNA encoding amino acids 1-165 of human kit ligand (SEQ ID NO:1) using PCR and the following primers: (SEQ ID NO:21): 5'-TCTAGAGTCCATATGGAAGGGATCTGC-3' and (SEQ ID NO:22): 5'-CGGGGTACCGGCTGCAACAGGGGGTAACAT-3'. A second construct, containing a HindIII and a BamHI site, respectively, at the front and back of SEQ ID NO:1 was created using PCR and the following primers:
(SEQ ID NO:23): 5'-AAGCTTGAAGGGATCAGGAATCGT-3' and
(SEQ ID NO:24): 5'-GGATCCTTACTAGGCTGCAACAGGGGG-3'.

The above two constructs were separately cloned into T vectors (Novagen T7 Blue) and sequenced. The KL sequences were then removed by digestion with the appropriate restriction enzymes (XbaI/KpnI or HindIII/BamHI) and cloned into the appropriate site of the same pGEM7Zf+ vector (Promega). The resultant vector was then cut with KpnI and HindIII to remove the sequences between the cloned inserts and ligated to the following pair of annealed linkers: (SEQ ID NO:25): 5'-CGGTGGCGGAGGGTCAGGTGGCGGAGGGTCGA-3' and (SEQ ID NO:26): 5'-AGCTTAGACCCTCCGCCACCTGACCCTCCGCCACCGGTC-3', to produce the cDNA depicted in SEQ ID NO:27. The above linkers encode the amino acid sequence Gly-Thr-$(Gly_4\text{-}Ser)_2$-Lys-Leu (amino acids 166-179 of SEQ ID NO:28).

To create a longer linker we ligated the following annealed linkers to the KpnI/HindIII cut vector: (SEQ ID NO:29):
5'CGGTGGCGGAGGGTCTGGTGGCGGAGGGTCCGGTGGAGGGTCAGGTGGCGGAGGGTCTA-3'
and (SEQ ID NO:30):
5'AGCTTAGACCCTCCGCCACCTGACCCTGACCCTCCGCCACCGGACCCTCCGCCACCAGACCCTC
CGCCACCGGTAC-3' to produce the cDNA depicted in SEQ ID NO:31. The above linkers encode the amino acid sequence Gly-Thr-$(Gly_4\text{-}Ser)_4$-Lys-Leu (amino acids 166-189 of SEQ ID NO:32).

After ligation of the appropriate annealed linkers, the DNA encoding the linker-linked dimer was excised out of the vector with NdeI and SacI and subcloned in to the expression vector pKK223-3. After transformation of an appropriate bacterial host, protein expression is induced by the addition of IPTG to a log phase culture. After 1 to 4 hours the bacteria are isolated and lysed by sonication. Inclusion bodies, if present, are also recovered. The dimers present in the lysate and inclusion bodies are further purified independently as previously described in Example 1.

The isolated, linker-linked KL dimers (SEQ ID NOS: 28 and 32) display greater cell proliferative activity than the monomeric forms, but no concomitant increase in mast cell activation.

Example 13: Formation of KL Heterodimers With Deleted Cysteines

A met-hKL cDNA having a Cys_{43}->Ser or a Cys_{138}->Ser mutation was created using the techniques described in Example 11 and the appropriate oligonucleotide. The resulting cDNAs (SEQ ID NOS: 33 and 35) are cloned into an expression vector under control of the P_L promoter. Following transformation of an appropriate bacterial host, protein is expressed, isolated and purified by ion exchange chromatography in the presence of urea. The isolated Cys_{43}->Ser and Cys_{138}->Ser KL monomers (SEQ ID NOS:34 and 36) are then combined in the presence of urea, fully reduced and the renatured under the conditions described in Example 1 for murine KL. Samples are taken 12, 24, 36 and 72 hours after refolding is initiated and chromatographed on a reverse phase HPLC column. Fractions are analyzed for biological activity and for the formation of disulfide linked dimers by reduced and non-reduced SDS PAGE.

The isolated, biologically active KL dimers display greater cell proliferative activity than the native or Cys-deleted monomeric forms, but no concomitant increase in mast cell activation.

Modifications and variations of the present invention will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CytoMed, Inc. (all states except US)
Nocka, Karl (US only)
Lobell, Robert B (US only)

(ii) TITLE OF INVENTION: STABILIZED DIMER OF KIT LIGAND AND FLT-3/FLK-2 LIGAND

(iii) NUMBER OF SEQUENCES: 36

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/220,379
(B) FILING DATE: 28-MAR-1994

(viii) ATTORNEY/AGENT INFORMATION:
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(B) TELEFAX: 212-596-9090

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..495
(D) OTHER INFORMATION: /product= "soluble human kit ligand (amino acids 1-165)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT GTA AAA GAC GTC ACT        48
Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val Thr
  1               5                  10                  15

AAA TTG GTG GCA AAT CTT CCA AAA GAC TAC ATG ATA ACC CTC AAA TAT        96
Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys Tyr
            20                  25                  30

GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT TGT TGG ATA AGC GAG ATG       144
Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu Met
        35                  40                  45

GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT CTG GAC AAG TTT TCA       192
Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe Ser
    50                  55                  60

AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC ATA GAC AAA CTT GTG       240
Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Val
65                  70                  75                  80

AAT ATA GTG GAT GAC CTT GTG GAG TGC GTG AAA GAA AAC TCA TCT AAG       288
Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser Lys
                85                  90                  95

GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC AGG CTC TTT ACT CCT       336
Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr Pro
            100                 105                 110

GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT GAT GCC TTC AAG GAC       384
Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp
        115                 120                 125

TTT GTA GTG GCA TCT GAA ACT AGT GAT TGT GTG GTT TCT TCA ACA TTA       432
Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr Leu
    130                 135                 140

AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA AAA CCA TTT ATG TTA       480
Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu
145                 150                 155                 160

CCC CCT GTT GCA GCC                                                  495
Pro Pro Val Ala Ala
                165
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val Thr
  1               5                  10                  15

Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys Tyr
            20                  25                  30

Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu Met
        35                  40                  45
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Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe Ser
     50                  55                  60

Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Val
 65                  70                  75                  80

Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser Lys
                85                  90                  95

Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr Pro
            100                 105                 110

Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp
        115                 120                 125

Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr Leu
    130                 135                 140

Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu
145                 150                 155                 160

Pro Pro Val Ala Ala
                165
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..495
(D) OTHER INFORMATION: /product= "murine KL (aa 1-165) souble form"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AAG GAG ATC TGC GGG AAT CCT GTG ACT GAT AAT GTA AAA GAC ATT ACA        48
Lys Glu Ile Cys Gly Asn Pro Val Thr Asp Asn Val Lys Asp Ile Thr
                170                 175                 180

AAA CTG GTG GCA AAT CTT CCA AAT GAC TAT ATG ATA ACC CTC AAC TAT        96
Lys Leu Val Ala Asn Leu Pro Asn Asp Tyr Met Ile Thr Leu Asn Tyr
            185                 190                 195

GTC GCC GGG ATG GAT GTT TTG CCT AGT CAT TGT TGG CTA CGA GAT ATG       144
Val Ala Gly Met Asp Val Leu Pro Ser His Cys Trp Leu Arg Asp Met
        200                 205                 210

GTA ATA CAA TTA TCA CTC AGC TTG ACT ACT CTT CTG GAC AAG TTC TCA       192
Val Ile Gln Leu Ser Leu Ser Leu Thr Thr Leu Leu Asp Lys Phe Ser
    215                 220                 225

AAT ATT TCT GAA GGC TTG AGT AAT TAC TCC ATC ATA GAC AAA CTT GGG       240
Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Gly
230                 235                 240                 245
```

```
AAA ATA GTG GAT GAC CTC GTG TTA TGC ATG GAA GAA AAC GCA CCG AAG       288
Lys Ile Val Asp Asp Leu Val Leu Cys Met Glu Glu Asn Ala Pro Lys
                250                 255                 260

AAT ATA AAA GAA TCT CCG AAG AGG CCA GAA ACT AGA TCC TTT ACT CCT       336
Asn Ile Lys Glu Ser Pro Lys Arg Pro Glu Thr Arg Ser Phe Thr Pro
            265                 270                 275

GAA GAA TTC TTT AGT ATT TTC AAT AGA TCC ATT GAT GCC TTT AAG GAC       384
Glu Glu Phe Phe Ser Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp
        280                 285                 290

TTT ATG GTG GCA TCT GAC ACT AGT GAC TGT GTG CTC TCT TCA ACA TTA       432
Phe Met Val Ala Ser Asp Thr Ser Asp Cys Val Leu Ser Ser Thr Leu
    295                 300                 305

GGT CCC GAG AAA GAT TCC AGA GTC AGT GTC ACA AAA CCA TTT ATG TTA       480
Gly Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu
310                 315                 320                 325

CCC CCT GTT GCA GCC                                                  495
Pro Pro Val Ala Ala
                330
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Lys Glu Ile Cys Gly Asn Pro Val Thr Asp Asn Val Lys Asp Ile Thr
  1               5                  10                  15

Lys Leu Val Ala Asn Leu Pro Asn Asp Tyr Met Ile Thr Leu Asn Tyr
            20                  25                  30

Val Ala Gly Met Asp Val Leu Pro Ser His Cys Trp Leu Arg Asp Met
        35                  40                  45

Val Ile Gln Leu Ser Leu Ser Leu Thr Thr Leu Leu Asp Lys Phe Ser
    50                  55                  60

Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Gly
65                  70                  75                  80

Lys Ile Val Asp Asp Leu Val Leu Cys Met Glu Glu Asn Ala Pro Lys
                85                  90                  95

Asn Ile Lys Glu Ser Pro Lys Arg Pro Glu Thr Arg Ser Phe Thr Pro
            100                 105                 110

Glu Glu Phe Phe Ser Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp
        115                 120                 125

Phe Met Val Ala Ser Asp Thr Ser Asp Cys Val Leu Ser Ser Thr Leu
    130                 135                 140

Gly Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu
145                 150                 155                 160

Pro Pro Val Ala Ala
```

165

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 165 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gln Glu Ile Cys Arg Asn Pro Val Thr Asp Asn Val Lys Asp Ile Thr
1 5 10 15

Lys Leu Val Ala Asn Leu Pro Asn Asp Tyr Met Ile Thr Leu Asn Tyr
20 25 30

Val Ala Gly Met Asp Val Leu Pro Ser His Cys Trp Leu Arg Asp Met
35 40 45

Val Thr His Leu Ser Val Ser Leu Thr Thr Leu Leu Asp Lys Phe Ser
50 55 60

Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Gly
65 70 75 80

Lys Ile Val Asp Asp Leu Val Ala Cys Met Glu Glu Asn Ala Pro Lys
85 90 95

Asn Val Lys Glu Ser Leu Lys Lys Pro Glu Thr Arg Asn Phe Thr Pro
100 105 110

Glu Glu Phe Phe Ser Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp
115 120 125

Phe Met Val Ala Ser Asp Thr Ser Asp Cys Val Leu Ser Ser Thr Leu
130 135 140

Gly Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu
145 150 155 160

Pro Pro Val Ala Ala
165

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 231 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..205

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Thr Val Leu Ala Pro Ala Trp Ser Pro Asn Ser Ser Leu Leu Leu
-25                 -20                 -15

Leu Leu Leu Leu Leu Ser Pro Cys Leu Arg Gly Thr Pro Asp Cys Tyr
-10                 -5                  1                   5

Phe Ser His Ser Pro Ile Ser Ser Asn Phe Lys Val Lys Phe Arg Glu
            10                  15                  20

Leu Thr Asp His Leu Leu Lys Asp Tyr Pro Val Thr Val Ala Val Asn
        25                  30                  35

Leu Gln Asp Glu Lys His Cys Lys Ala Leu Trp Ser Leu Phe Leu Ala
    40                  45                  50

Gln Arg Trp Ile Glu Gln Leu Lys Thr Val Ala Gly Ser Lys Met Gln
55                  60                  65                  70

Thr Leu Leu Glu Asp Val Asn Thr Glu Ile His Phe Val Thr Ser Cys
                75                  80                  85

Thr Phe Gln Pro Leu Pro Glu Cys Leu Arg Phe Val Gln Thr Asn Ile
            90                  95                  100

Ser His Leu Leu Lys Asp Thr Cys Thr Gln Leu Leu Ala Leu Lys Pro
        105                 110                 115

Cys Ile Gly Lys Ala Cys Gln Asn Phe Ser Arg Cys Leu Glu Val Gln
    120                 125                 130

Cys Gln Pro Asp Ser Ser Thr Leu Leu Pro Pro Arg Ser Pro Ile Ala
135                 140                 145                 150

Leu Glu Ala Thr Glu Leu Pro Glu Pro Arg Pro Arg Gln Leu Leu Leu
                155                 160                 165

Leu Leu Leu Leu Leu Pro Leu Thr Leu Val Leu Leu Ala Ala Ala Trp
            170                 175                 180

Gly Leu Arg Trp Gln Arg Ala Arg Arg Lys Gly Glu Leu His Pro Gly
        185                 190                 195

Val Pro Leu Pro Ser His Pro
    200                 205
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1275 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1275

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 1..75

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 76..1275
(D) OTHER INFORMATION: /product= "murine KL_Ig fusion protein"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATG AAG AAG ACA CAA ACT TGG ATT ATC ACT TGC ATT TAT CTT CAA CTG        48
Met Lys Lys Thr Gln Thr Trp Ile Ile Thr Cys Ile Tyr Leu Gln Leu
-25                 -20                 -15                 -10

CTC CTA TTT AAT CCT CTT GTC AAA ACC AAG GAG ATC TGC GGG AAT CCT        96
Leu Leu Phe Asn Pro Leu Val Lys Thr Lys Glu Ile Cys Gly Asn Pro
                -5                  1                   5

GTG ACT GAT AAT GTA AAA GAC ATT ACA AAA CTG GTG GCA AAT CTT CCA       144
Val Thr Asp Asn Val Lys Asp Ile Thr Lys Leu Val Ala Asn Leu Pro
        10                  15                  20

AAT GAC TAT ATG ATA ACC CTC AAC TAT GTC GCC GGG ATG GAT GTT TTG       192
Asn Asp Tyr Met Ile Thr Leu Asn Tyr Val Ala Gly Met Asp Val Leu
    25                  30                  35

CCT AGT CAT TGT TGG CTA CGA GAT ATG GTA ATA CAA TTA TCA CTC AGC       240
Pro Ser His Cys Trp Leu Arg Asp Met Val Ile Gln Leu Ser Leu Ser
40                  45                  50                  55

TTG ACT ACT CTT CTG GAC AAG TTC TCA AAT ATT TCT GAA GGC TTG AGT       288
Leu Thr Thr Leu Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser
                60                  65                  70

AAT TAC TCC ATC ATA GAC AAA CTT GGG AAA ATA GTG GAT GAC CTC GTG       336
Asn Tyr Ser Ile Ile Asp Lys Leu Gly Lys Ile Val Asp Asp Leu Val
            75                  80                  85

TTA TGC ATG GAA GAA AAC GCA CCG AAG AAT ATA AAA GAA TCT CCG AAG       384
Leu Cys Met Glu Glu Asn Ala Pro Lys Asn Ile Lys Glu Ser Pro Lys
        90                  95                  100

AGG CCA GAA ACT AGA TCC TTT ACT CCT GAA GAA TTC TTT AGT ATT TTC       432
Arg Pro Glu Thr Arg Ser Phe Thr Pro Glu Glu Phe Phe Ser Ile Phe
    105                 110                 115

AAT AGA TCC ATT GAT GCC TTT AAG GAC TTT ATG GTG GCA TCT GAC ACT       480
Asn Arg Ser Ile Asp Ala Phe Lys Asp Phe Met Val Ala Ser Asp Thr
120                 125                 130                 135

AGT GAC TGT GTG CTC TCT TCA ACA TTA GGT CCC GAG AAA GAT TCC AGA       528
Ser Asp Cys Val Leu Ser Ser Thr Leu Gly Pro Glu Lys Asp Ser Arg
                140                 145                 150

GTC AGT GTC ACA AAA CCA TTT ATG TTA CCC CCT GTT GCA GCC GAT CCC       576
Val Ser Val Thr Lys Pro Phe Met Leu Pro Pro Val Ala Ala Asp Pro
            155                 160                 165

GAG CCC AGA GGG CCC ACA ATC AAG CCC TGT CCT CCA TGC AAA TGC CCA       624
Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro
```

```
        170                 175                 180

GCA CCT AAC CTC TTG GGT GGA CCA TCC GTC TTC ATC TTC CCT CCA AAG      672
Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
    185                 190                 195

ATC AAG GAT GTA CTC ATG ATC TCC CTG AGC CCC ATA GTC ACA TGT GTG      720
Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val
200                 205                 210                 215

GTG GTG GAT GTG AGC GAG GAT GAC CCA GAT GTC CAG ATC AGC TGG TTT      768
Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe
                220                 225                 230

GTG AAC AAC GTG GAA GTA CAC ACA GCT CAG ACA CAA ACC CAT AGA GAG      816
Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu
            235                 240                 245

GAT TAC AAC AGT ACT CTC CGG GTG GTC AGT GCC CTC CCC ATC CAG CAC      864
Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His
        250                 255                 260

CAG GAC TGG ATG AGT GGC AAG GAG TTC AAA TGC AAG GTC AAC AAC AAA      912
Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys
    265                 270                 275

GAC CTG CCA GCG CCC ATC GAG AGA ACC ATC TCA AAA CCC AAA GGG TCA      960
Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser
280                 285                 290                 295

GTA AGA GCT CCA CAG GTA TAT GTC TTG CCT CCA CCA GAA GAA GAG ATG     1008
Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met
                300                 305                 310

ACT AAG AAA CAG GTC ACT CTG ACC TGC ATG GTC ACA GAC TTC ATG CCT     1056
Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro
            315                 320                 325

GAA GAC ATT TAC GTG GAG TGG ACC AAC AAC GGG AAA ACA GAG CTA AAC     1104
Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn
        330                 335                 340

TAC AAG AAC ACT GAA CCA GTC CTG GAC TCT GAT GGT TCT TAC TTC ATG     1152
Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met
    345                 350                 355

TAC AGC AAG CTG AGA GTG GAA AAG AAG AAC TGG GTG GAA AGA AAT AGC     1200
Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser
360                 365                 370                 375

TAC TCC TGT TCA GTG GTC CAC GAG GGT CTG CAC AAT CAC CAC ACG ACT     1248
Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr
                380                 385                 390

AAG AGC TTC TCC CGG ACT CCG GGT AAA                                 1275
Lys Ser Phe Ser Arg Thr Pro Gly Lys
            395                 400
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 425 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Lys Lys Thr Gln Thr Trp Ile Ile Thr Cys Ile Tyr Leu Gln Leu
-25                 -20                 -15                 -10

Leu Leu Phe Asn Pro Leu Val Lys Thr Lys Glu Ile Cys Gly Asn Pro
                -5                  1                   5

Val Thr Asp Asn Val Lys Asp Ile Thr Lys Leu Val Ala Asn Leu Pro
        10                  15                  20

Asn Asp Tyr Met Ile Thr Leu Asn Tyr Val Ala Gly Met Asp Val Leu
    25                  30                  35

Pro Ser His Cys Trp Leu Arg Asp Met Val Ile Gln Leu Ser Leu Ser
40                  45                  50                  55

Leu Thr Thr Leu Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser
                60                  65                  70

Asn Tyr Ser Ile Ile Asp Lys Leu Gly Lys Ile Val Asp Asp Leu Val
            75                  80                  85

Leu Cys Met Glu Glu Asn Ala Pro Lys Asn Ile Lys Glu Ser Pro Lys
        90                  95                  100

Arg Pro Glu Thr Arg Ser Phe Thr Pro Glu Glu Phe Phe Ser Ile Phe
    105                 110                 115

Asn Arg Ser Ile Asp Ala Phe Lys Asp Phe Met Val Ala Ser Asp Thr
120                 125                 130                 135

Ser Asp Cys Val Leu Ser Ser Thr Leu Gly Pro Glu Lys Asp Ser Arg
                140                 145                 150

Val Ser Val Thr Lys Pro Phe Met Leu Pro Pro Val Ala Ala Asp Pro
            155                 160                 165

Glu Pro Arg Gly Pro Thr Ile Lys Pro Cys Pro Pro Cys Lys Cys Pro
        170                 175                 180

Ala Pro Asn Leu Leu Gly Gly Pro Ser Val Phe Ile Phe Pro Pro Lys
    185                 190                 195

Ile Lys Asp Val Leu Met Ile Ser Leu Ser Pro Ile Val Thr Cys Val
200                 205                 210                 215

Val Val Asp Val Ser Glu Asp Asp Pro Asp Val Gln Ile Ser Trp Phe
                220                 225                 230

Val Asn Asn Val Glu Val His Thr Ala Gln Thr Gln Thr His Arg Glu
            235                 240                 245

Asp Tyr Asn Ser Thr Leu Arg Val Val Ser Ala Leu Pro Ile Gln His
        250                 255                 260

Gln Asp Trp Met Ser Gly Lys Glu Phe Lys Cys Lys Val Asn Asn Lys
    265                 270                 275

Asp Leu Pro Ala Pro Ile Glu Arg Thr Ile Ser Lys Pro Lys Gly Ser
280                 285                 290                 295

Val Arg Ala Pro Gln Val Tyr Val Leu Pro Pro Pro Glu Glu Glu Met
                300                 305                 310

Thr Lys Lys Gln Val Thr Leu Thr Cys Met Val Thr Asp Phe Met Pro
            315                 320                 325
```

Glu Asp Ile Tyr Val Glu Trp Thr Asn Asn Gly Lys Thr Glu Leu Asn
330 335 340

Tyr Lys Asn Thr Glu Pro Val Leu Asp Ser Asp Gly Ser Tyr Phe Met
345 350 355

Tyr Ser Lys Leu Arg Val Glu Lys Lys Asn Trp Val Glu Arg Asn Ser
360 365 370 375

Tyr Ser Cys Ser Val Val His Glu Gly Leu His Asn His His Thr Thr
380 385 390

Lys Ser Phe Ser Arg Thr Pro Gly Lys
395 400

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACTCGAGCC ACCAATGAAG AAGACACAAA CTTGG 35

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCAGGGATCC GCTGCAACAG GGGGTAACAT AAA 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1272 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 76..1272
(D) OTHER INFORMATION: /product= "human KL-Ig fusion protein"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1272

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
ATG AAG AAG ACA CAA ACT TGG ATT CTC ACT TGC ATT TAT CTT CAG CTG        48
Met Lys Lys Thr Gln Thr Trp Ile Leu Thr Cys Ile Tyr Leu Gln Leu
-25                 -20                 -15                 -10

CTC CTA TTT AAT CCT CTC GTC AAA ACT GAA GGG ATC TGC AGG AAT CGT        96
Leu Leu Phe Asn Pro Leu Val Lys Thr Glu Gly Ile Cys Arg Asn Arg
                -5                   1                   5

GTG ACT AAT AAT GTA AAA GAC GTC ACT AAA TTG GTG GCA AAT CTT CCA       144
Val Thr Asn Asn Val Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro
        10                  15                  20

AAG GAC TAC ATG ATA ACC CTC AAA TAT GTC CCC GGG ATG GAT ATT TTG       192
Lys Asp Tyr Met Ile Thr Leu Lys Tyr Val Pro Gly Met Asp Ile Leu
     25                  30                  35

CCA AGT CAT TGT TGG ATA AGC GAG ATG GTA GTA CAA TTG TCA GAC AGC       240
Pro Ser His Cys Trp Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser
 40                  45                  50                  55

TTG ACT GAT CTT CTG GAC AAG TTT TCA AAT ATT TCT GAA GGC TTG AGT       288
Leu Thr Asp Leu Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser
                60                  65                  70

AAT TAT TCC ATC ATA GAC AAA CTT GTG AAT ATA GTG GAT GAC CTT GTG       336
Asn Tyr Ser Ile Ile Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val
            75                  80                  85

GAG TGC GTG AAA GAA AAC TCA TCT AAG GAT CTA AAA AAA TCA TTC AAG       384
Glu Cys Val Lys Glu Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys
        90                  95                  100

AGC CCA GAA CCC AGG CTC TTT ACT CCT GAA GAA TTC TTT AGA ATT TTT       432
Ser Pro Glu Pro Arg Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe
    105                 110                 115

AAT AGA TCC ATT GAT GCC TTC AAG GAC TTT GTA GTG GCA TCT GAA ACT       480
Asn Arg Ser Ile Asp Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr
120                 125                 130                 135

AGT GAT TGT GTG GTT TCT TCA ACA TTA AGT CCT GAG AAA GAT TCC AGA       528
Ser Asp Cys Val Val Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg
                140                 145                 150

GTC AGT GTC ACA AAA CCA TTT ATG TTA CCC CCT GTT GCA GCG GAT CCC       576
Val Ser Val Thr Lys Pro Phe Met Leu Pro Pro Val Ala Ala Asp Pro
            155                 160                 165
```

```
GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA        624
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        170                 175                 180

CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC        672
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    185                 190                 195

AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG        720
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
200                 205                 210                 215

GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG        768
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                220                 225                 230

GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG        816
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            235                 240                 245

TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG        864
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        250                 255                 260

GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC        912
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    265                 270                 275

CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC        960
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
280                 285                 290                 295

CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC       1008
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
                300                 305                 310

AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC       1056
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            315                 320                 325

GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC       1104
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        330                 335                 340

AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC       1152
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    345                 350                 355

AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC       1200
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
360                 365                 370                 375

TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG       1248
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                380                 385                 390

AGC CTC TCC CTG TCT CCG GGT AAA                                      1272
Ser Leu Ser Leu Ser Pro Gly Lys
            395
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 424 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Lys Lys Thr Gln Thr Trp Ile Leu Thr Cys Ile Tyr Leu Gln Leu
-25                 -20                 -15                 -10

Leu Leu Phe Asn Pro Leu Val Lys Thr Glu Gly Ile Cys Arg Asn Arg
                -5                  1                   5

Val Thr Asn Asn Val Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro
        10                  15                  20

Lys Asp Tyr Met Ile Thr Leu Lys Tyr Val Pro Gly Met Asp Ile Leu
    25                  30                  35

Pro Ser His Cys Trp Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser
40                  45                  50                  55

Leu Thr Asp Leu Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser
                60                  65                  70

Asn Tyr Ser Ile Ile Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val
            75                  80                  85

Glu Cys Val Lys Glu Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys
        90                  95                  100

Ser Pro Glu Pro Arg Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe
    105                 110                 115

Asn Arg Ser Ile Asp Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr
120                 125                 130                 135

Ser Asp Cys Val Val Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg
                140                 145                 150

Val Ser Val Thr Lys Pro Phe Met Leu Pro Pro Val Ala Ala Asp Pro
            155                 160                 165

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        170                 175                 180

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    185                 190                 195

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
200                 205                 210                 215

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                220                 225                 230

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            235                 240                 245

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        250                 255                 260

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    265                 270                 275

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
280                 285                 290                 295

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
                300                 305                 310
```

```
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            315                 320                 325

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        330                 335                 340

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    345                 350                 355

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
360                 365                 370                 375

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                380                 385                 390

Ser Leu Ser Leu Ser Pro Gly Lys
            395
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1272 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 76..1272
(D) OTHER INFORMATION: /product= "human KL-Ig fusion protein"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1272

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
ATG AAG AAG ACA CAA ACT TGG ATT CTC ACT TGC ATT TAT CTT CAG CTG        48
Met Lys Lys Thr Gln Thr Trp Ile Leu Thr Cys Ile Tyr Leu Gln Leu
-25                 -20                 -15                 -10

CTC CTA TTT AAT CCT CTC GTC AAA ACT GAA GGG ATC TGC AGG AAT CGT        96
Leu Leu Phe Asn Pro Leu Val Lys Thr Glu Gly Ile Cys Arg Asn Arg
                -5                  1                   5

GTG ACT AAT AAT GTA AAA GAC GTC ACT AAA TTG GTG GCA AAT CTT CCA       144
Val Thr Asn Asn Val Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro
        10                  15                  20

AAG GAC TAC ATG ATA ACC CTC AAA TAT GTC CCC GGG ATG GAT GTT TTG       192
Lys Asp Tyr Met Ile Thr Leu Lys Tyr Val Pro Gly Met Asp Val Leu
    25                  30                  35

CCA AGT CAT TGT TGG ATA AGC GAG ATG GTA GTA CAA TTG TCA GAC AGC       240
Pro Ser His Cys Trp Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser
40                  45                  50                  55

TTG ACT GAT CTT CTG GAC AAG TTT TCA AAT ATT TCT GAA GGC TTG AGT       288
```

```
Leu Thr Asp Leu Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser
                60                  65                  70

AAT TAT TCC ATC ATA GAC AAA CTT GTG AAT ATA GTG GAT GAC CTT GTG      336
Asn Tyr Ser Ile Ile Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val
            75                  80                  85

GAG TGC GTG AAA GAA AAC TCA TCT AAG GAT CTA AAA AAA TCA TTC AAG      384
Glu Cys Val Lys Glu Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys
        90                  95                 100

AGC CCA GAA CCC AGG CTC TTT ACT CCT GAA GAA TTC TTT AGA ATT TTT      432
Ser Pro Glu Pro Arg Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe
    105                 110                 115

AAT AGA TCC ATT GAT GCC TTC AAG GAC TTT GTA GTG GCA TCT GAA ACT      480
Asn Arg Ser Ile Asp Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr
120                 125                 130                 135

AGT GAT TGT GTG GTT TCT TCA ACA TTA AGT CCT GAG AAA GAT TCC AGA      528
Ser Asp Cys Val Val Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg
                140                 145                 150

GTC AGT GTC ACA AAA CCA TTT ATG TTA CCC CCT GTT GCA GCG GAT CCC      576
Val Ser Val Thr Lys Pro Phe Met Leu Pro Pro Val Ala Ala Asp Pro
            155                 160                 165

GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA      624
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        170                 175                 180

CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC      672
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    185                 190                 195

AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG      720
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
200                 205                 210                 215

GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG      768
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                220                 225                 230

GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG      816
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            235                 240                 245

TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG      864
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        250                 255                 260

GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC      912
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    265                 270                 275

CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC      960
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
280                 285                 290                 295

CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC     1008
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
                300                 305                 310

AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC     1056
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            315                 320                 325
```

```
GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC      1104
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        330                 335                 340

AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC      1152
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    345                 350                 355

AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC      1200
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
360                 365                 370                 375

TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG      1248
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                380                 385                 390

AGC CTC TCC CTG TCT CCG GGT AAA                                     1272
Ser Leu Ser Leu Ser Pro Gly Lys
            395
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 424 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Lys Lys Thr Gln Thr Trp Ile Leu Thr Cys Ile Tyr Leu Gln Leu
-25                 -20                 -15                 -10

Leu Leu Phe Asn Pro Leu Val Lys Thr Glu Gly Ile Cys Arg Asn Arg
                -5                  1                   5

Val Thr Asn Asn Val Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro
        10                  15                  20

Lys Asp Tyr Met Ile Thr Leu Lys Tyr Val Pro Gly Met Asp Val Leu
    25                  30                  35

Pro Ser His Cys Trp Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser
40                  45                  50                  55

Leu Thr Asp Leu Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser
                60                  65                  70

Asn Tyr Ser Ile Ile Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val
            75                  80                  85

Glu Cys Val Lys Glu Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys
        90                  95                  100

Ser Pro Glu Pro Arg Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe
    105                 110                 115

Asn Arg Ser Ile Asp Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr
120                 125                 130                 135

Ser Asp Cys Val Val Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg
                140                 145                 150

Val Ser Val Thr Lys Pro Phe Met Leu Pro Pro Val Ala Ala Asp Pro
            155                 160                 165
```

```
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        170                 175                 180

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    185                 190                 195

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
200                 205                 210                 215

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                220                 225                 230

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            235                 240                 245

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        250                 255                 260

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    265                 270                 275

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
280                 285                 290                 295

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
                300                 305                 310

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            315                 320                 325

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        330                 335                 340

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    345                 350                 355

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
360                 365                 370                 375

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                380                 385                 390

Ser Leu Ser Leu Ser Pro Gly Lys
            395
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAGGGTTATC ATGCAGTCTT TTGGAAG 27

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucloetide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAGGGTTATC ATGCAGTGTC TTTTGG 26

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 498 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..498

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 4..498
(D) OTHER INFORMATION: /product= "human KL w/Tyr->Cys substitution at aa 26"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
ATG GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT GTA AAA GAC GTC        48
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1               5                  10                  15

ACT AAA TTG GTG GCA AAT CTT CCA AAA GAC TGC ATG ATA ACC CTC AAA        96
Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Cys Met Ile Thr Leu Lys
                20                  25                  30

TAT GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT TGT TGG ATA AGC GAG       144
Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu
            35                  40                  45

ATG GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT CTG GAC AAG TTT       192
Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

TCA AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC ATA GAC AAA CTT       240
```

```
Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
 65                  70                  75

GTG AAT ATA GTC GAT GAC CTT GTG GAG TGC GTC AAA GAA AAC TCA TCT      288
Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
 80                  85                  90                  95

AAG GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC AGG CTC TTT ACT      336
Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110

CCT GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT GAT GCC TTC AAG      384
Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
            115                 120                 125

GAC TTT GTA GTG GCA TCT GAA ACT AGT GAT TGT GTG GTT TCT TCA ACA      432
Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr
        130                 135                 140

TTA AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA AAA CCA TTT ATG      480
Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
    145                 150                 155

TTA CCC CCT GTT GCA GCC                                             498
Leu Pro Pro Val Ala Ala
160                 165
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1                   5                  10                  15

Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Cys Met Ile Thr Leu Lys
                20                  25                  30

Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu
            35                  40                  45

Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
 65                  70                  75

Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
 80                  85                  90                  95

Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110

Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
            115                 120                 125

Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr
        130                 135                 140

Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
```

```
    145                 150                 155

Leu Pro Pro Val Ala Ala
160                 165
```

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 501 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..501

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 4..501
(D) OTHER INFORMATION: /product= "human KL w/extra Cys inserted at aa 27"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
ATG GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT GTA AAA GAC GTC          48
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1                5                  10                  15

ACT AAA TTG GTG GCA AAT CTT CCA AAA GAC TAC TGC ATG ATA ACC CTC          96
Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Cys Met Ile Thr Leu
                20                  25                  30

AAA TAT GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT TGT TGG ATA AGC         144
Lys Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser
            35                  40                  45

GAG ATG GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT CTG GAC AAG         192
Glu Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys
        50                  55                  60

TTT TCA AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC ATA GAC AAA         240
Phe Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys
    65                  70                  75

CTT GTG AAT ATA GTC GAT GAC CTT GTG GAG TGC GTC AAA GAA AAC TCA         288
Leu Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser
80                  85                  90                  95

TCT AAG GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC AGG CTC TTT         336
Ser Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe
                100                 105                 110

ACT CCT GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT GAT GCC TTC         384
Thr Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe
            115                 120                 125

AAG GAC TTT GTA GTG GCA TCT GAA ACT AGT GAT TGT GTG GTT TCT TCA         432
Lys Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser
```

```
        130                 135                 140

ACA TTA AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA AAA CCA TTT      480
Thr Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe
    145                 150                 155

ATG TTA CCC CCT GTT GCA GCC                                      501
Met Leu Pro Pro Val Ala Ala
160                 165
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 167 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1               5                  10                  15

Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Cys Met Ile Thr Leu
                20                  25                  30

Lys Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser
            35                  40                  45

Glu Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys
        50                  55                  60

Phe Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys
    65                  70                  75

Leu Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser
80                  85                  90                  95

Ser Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe
                100                 105                 110

Thr Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe
            115                 120                 125

Lys Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser
        130                 135                 140

Thr Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe
    145                 150                 155

Met Leu Pro Pro Val Ala Ala
160                 165
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCTAGAGTCC ATATGGAAGG GATCTGC 27

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGGGTACCG GCTGCAACAG GGGGTAACAT 30

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AAGCTTGAAG GGATCAGGAA TCGT 24

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGATCCTTAC TAGGCTGCAA CAGGGGG 27

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CGGTGGCGGA GGGTCAGGTG GCGGAGGGTC GA 32

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AGCTTAGACC CTCCGCCACC TGACCCTCCG CCACCGGTC 39

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1059 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 13..1050
(D) OTHER INFORMATION: /product= "human KL fusion dimer with linker"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 16..1050

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
TCTAGAGTCC AT ATG GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT          48
              Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn
               -1   1               5                  10

GTA AAA GAC GTC ACT AAA TTG GTG GCA AAT CTT CCA AAA GAC TAC ATG        96
Val Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met
            15                  20                  25

ATA ACC CTC AAA TAT GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT TGT       144
Ile Thr Leu Lys Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys
        30                  35                  40

TGG ATA AGC GAG ATG GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT       192
Trp Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu
    45                  50                  55

CTG GAC AAG TTT TCA AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC       240
Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile
60                  65                  70                  75

ATA GAC AAA CTT GTG AAT ATA GTC GAT GAC CTT GTG GAG TGC GTC AAA       288
Ile Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys
                80                  85                  90

GAA AAC TCA TCT AAG GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC       336
Glu Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro
            95                  100                 105

AGG CTC TTT ACT CCT GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT       384
Arg Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile
        110                 115                 120

GAT GCC TTC AAG GAC TTT GTA GTG GCA TCT GAA ACT AGT GAT TGT GTG       432
Asp Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val
    125                 130                 135

GTT TCT TCA ACA TTA AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA       480
Val Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr
140                 145                 150                 155

AAA CCA TTT ATG TTA CCC CCT GTT GCA GCC GGT ACC GGT GGC GGA GGG       528
Lys Pro Phe Met Leu Pro Pro Val Ala Ala Gly Thr Gly Gly Gly Gly
                160                 165                 170

TCA GGT GGC GGA GGG TCT AAG CTT GAA GGG ATC TGC AGG AAT CGT GTG       576
Ser Gly Gly Gly Gly Ser Lys Leu Glu Gly Ile Cys Arg Asn Arg Val
            175                 180                 185

ACT AAT AAT GTA AAA GAC GTC ACT AAA TTG GTG GCA AAT CTT CCA AAA       624
Thr Asn Asn Val Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro Lys
        190                 195                 200

GAC TAC ATG ATA ACC CTC AAA TAT GTC CCC GGG ATG GAT GTT TTG CCA       672
Asp Tyr Met Ile Thr Leu Lys Tyr Val Pro Gly Met Asp Val Leu Pro
    205                 210                 215
```

```
AGT CAT TGT TGG ATA AGC GAG ATG GTA GTA CAA TTG TCA GAC AGC TTG        720
Ser His Cys Trp Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser Leu
220                 225                 230                 235

ACT GAT CTT CTG GAC AAG TTT TCA AAT ATT TCT GAA GGC TTG AGT AAT        768
Thr Asp Leu Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser Asn
                240                 245                 250

TAT TCC ATC ATA GAC AAA CTT GTG AAT ATA GTC GAT GAC CTT GTG GAG        816
Tyr Ser Ile Ile Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val Glu
            255                 260                 265

TGC GTC AAA GAA AAC TCA TCT AAG GAT CTA AAA AAA TCA TTC AAG AGC        864
Cys Val Lys Glu Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys Ser
        270                 275                 280

CCA GAA CCC AGG CTC TTT ACT CCT GAA GAA TTC TTT AGA ATT TTT AAT        912
Pro Glu Pro Arg Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe Asn
    285                 290                 295

AGA TCC ATT GAT GCC TTC AAG GAC TTT GTA GTG GCA TCT GAA ACT AGT        960
Arg Ser Ile Asp Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr Ser
300                 305                 310                 315

GAT TGT GTG GTT TCT TCA ACA TTA AGT CCT GAG AAA GAT TCC AGA GTC       1008
Asp Cys Val Val Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg Val
                320                 325                 330

AGT GTC ACA AAA CCA TTT ATG TTA CCC CCT GTT GCA GCC TAG               1050
Ser Val Thr Lys Pro Phe Met Leu Pro Pro Val Ala Ala  *
            335                 340                 345

TAAGGATCC                                                            1059
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 346 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1                5                  10                  15

Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys
                20                  25                  30

Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu
            35                  40                  45

Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
    65                  70                  75

Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
 80                  85                  90                  95

Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110
```

```
Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
           115                 120                 125

Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr
       130                 135                 140

Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
   145                 150                 155

Leu Pro Pro Val Ala Ala Gly Thr Gly Gly Gly Gly Ser Gly Gly Gly
160                 165                 170                 175

Gly Ser Lys Leu Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val
                180                 185                 190

Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile
           195                 200                 205

Thr Leu Lys Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp
       210                 215                 220

Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu
   225                 230                 235

Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile
240                 245                 250                 255

Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu
                260                 265                 270

Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg
           275                 280                 285

Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp
       290                 295                 300

Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val
   305                 310                 315

Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys
320                 325                 330                 335

Pro Phe Met Leu Pro Pro Val Ala Ala  *
                340                 345
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
CGGTGGCGGA GGGTCTGGTG GCGGAGGGTC CGGTGGAGGG TCAGGTGGCG GAGGGTCTA    59
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGCTTAGACC CTCCGCCACC TGACCCTGAC CCTCCGCCAC CGGACCCTCC GCCACCAGAC 60

CCTCCGCCAC CGGTAC 76

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1089 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 13..1080
(D) OTHER INFORMATION: /product= "human KL fusion dimer with linker"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 16..1080

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
TCTAGAGTCC AT ATG GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT          48
              Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn
               -1   1               5                  10

GTA AAA GAC GTC ACT AAA TTG GTG GCA AAT CTT CCA AAA GAC TAC ATG        96
Val Lys Asp Val Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met
            15                  20                  25

ATA ACC CTC AAA TAT GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT TGT       144
Ile Thr Leu Lys Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys
         30                  35                  40

TGG ATA AGC GAG ATG GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT       192
Trp Ile Ser Glu Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu
     45                  50                  55
```

```
CTG GAC AAG TTT TCA AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC        240
Leu Asp Lys Phe Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile
 60                  65                  70                  75

ATA GAC AAA CTT GTG AAT ATA GTC GAT GAC CTT GTG GAG TGC GTC AAA        288
Ile Asp Lys Leu Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys
                 80                  85                  90

GAA AAC TCA TCT AAG GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC        336
Glu Asn Ser Ser Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro
            95                 100                 105

AGG CTC TTT ACT CCT GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT        384
Arg Leu Phe Thr Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile
        110                 115                 120

GAT GCC TTC AAG GAC TTT GTA GTG GCA TCT GAA ACT AGT GAT TGT GTG        432
Asp Ala Phe Lys Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val
    125                 130                 135

GTT TCT TCA ACA TTA AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA        480
Val Ser Ser Thr Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr
140                 145                 150                 155

AAA CCA TTT ATG TTA CCC CCT GTT GCA GCC GGT ACC GGT GGC GGA GGG        528
Lys Pro Phe Met Leu Pro Pro Val Ala Ala Gly Thr Gly Gly Gly Gly
                160                 165                 170

TCT GGT GGC GGA GGG TCC GGT GGC GGA GGG TCA GGT GGC GGA GGG TCT        576
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
            175                 180                 185

AAG CTT GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT GTA AAA GAC        624
Lys Leu Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp
        190                 195                 200

GTC ACT AAA TTG GTG GCA AAT CTT CCA AAA GAC TAC ATG ATA ACC CTC        672
Val Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu
    205                 210                 215

AAA TAT GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT TGT TGG ATA AGC        720
Lys Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser
220                 225                 230                 235

GAG ATG GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT CTG GAC AAG        768
Glu Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys
                240                 245                 250

TTT TCA AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC ATA GAC AAA        816
Phe Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys
            255                 260                 265

CTT GTG AAT ATA GTC GAT GAC CTT GTG GAG TGC GTC AAA GAA AAC TCA        864
Leu Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser
        270                 275                 280

TCT AAG GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC AGG CTC TTT        912
Ser Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe
    285                 290                 295

ACT CCT GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT GAT GCC TTC        960
Thr Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe
300                 305                 310                 315

AAG GAC TTT GTA GTG GCA TCT GAA ACT AGT GAT TGT GTG GTT TCT TCA       1008
Lys Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser
                320                 325                 330
```

```
ACA TTA AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA AAA CCA TTT      1056
Thr Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe
            335                 340                 345

ATG TTA CCC CCT GTT GCA GCC TAG TAAGGATCC                          1089
Met Leu Pro Pro Val Ala Ala  *
        350                 355
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 356 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1               5                  10                  15

Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys
                20                  25                  30

Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu
            35                  40                  45

Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
    65                  70                  75

Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
80                  85                  90                  95

Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110

Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
            115                 120                 125

Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr
        130                 135                 140

Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
    145                 150                 155

Leu Pro Pro Val Ala Ala Gly Thr Gly Gly Gly Gly Ser Gly Gly Gly
160                 165                 170                 175

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Lys Leu Glu Gly
                180                 185                 190

Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val Thr Lys Leu
            195                 200                 205

Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys Tyr Val Pro
        210                 215                 220

Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu Met Val Val
    225                 230                 235

Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe Ser Asn Ile
240                 245                 250                 255
```

```
Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu Val Asn Ile
                260                 265                 270

Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser Lys Asp Leu
            275                 280                 285

Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr Pro Glu Glu
        290                 295                 300

Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys Asp Phe Val
    305                 310                 315

Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr Leu Ser Pro
320                 325                 330                 335

Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met Leu Pro Pro
                340                 345                 350

Val Ala Ala  *
            355
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 498 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..498

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 4..498
(D) OTHER INFORMATION: /product= "human KL with Cys->Ser substitution at aa 43"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
ATG GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT GTA AAA GAC GTC        48
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1               5                  10                  15

ACT AAA TTG GTG GCA AAT CTT CCA AAA GAC TAC ATG ATA ACC CTC AAA        96
Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys
                20                  25                  30

TAT GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT AGT TGG ATA AGC GAG       144
Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Ser Trp Ile Ser Glu
            35                  40                  45

ATG GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT CTG GAC AAG TTT       192
Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

TCA AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC ATA GAC AAA CTT       240
Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
```

```
     65                  70                  75

GTG AAT ATA GTC GAT GAC CTT GTG GAG TGC GTC AAA GAA AAC TCA TCT        288
Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
 80                  85                  90                  95

AAG GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC AGG CTC TTT ACT        336
Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110

CCT GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT GAT GCC TTC AAG        384
Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
            115                 120                 125

GAC TTT GTA GTG GCA TCT GAA ACT AGT GAT TGT GTG GTT TCT TCA ACA        432
Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr
        130                 135                 140

TTA AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA AAA CCA TTT ATG        480
Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
    145                 150                 155

TTA CCC CCT GTT GCA GCC                                               498
Leu Pro Pro Val Ala Ala
160                 165
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1                   5                  10                  15

Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys
                20                  25                  30

Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Ser Trp Ile Ser Glu
            35                  40                  45

Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
    65                  70                  75

Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
 80                  85                  90                  95

Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110

Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
            115                 120                 125

Asp Phe Val Val Ala Ser Glu Thr Ser Asp Cys Val Val Ser Ser Thr
        130                 135                 140

Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
    145                 150                 155
```

```
Leu Pro Pro Val Ala Ala
160                 165
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 498 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..498

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 4..498
(D) OTHER INFORMATION: /product= "human KL with Cys->Ser substitution at aa 138"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
ATG GAA GGG ATC TGC AGG AAT CGT GTG ACT AAT AAT GTA AAA GAC GTC        48
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1               5                  10                  15

ACT AAA TTG GTG GCA AAT CTT CCA AAA GAC TAC ATG ATA ACC CTC AAA        96
Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys
                20                  25                  30

TAT GTC CCC GGG ATG GAT GTT TTG CCA AGT CAT TGT TGG ATA AGC GAG       144
Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu
            35                  40                  45

ATG GTA GTA CAA TTG TCA GAC AGC TTG ACT GAT CTT CTG GAC AAG TTT       192
Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

TCA AAT ATT TCT GAA GGC TTG AGT AAT TAT TCC ATC ATA GAC AAA CTT       240
Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
    65                  70                  75

GTG AAT ATA GTC GAT GAC CTT GTG GAG TGC GTC AAA GAA AAC TCA TCT       288
Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
80                  85                  90                  95

AAG GAT CTA AAA AAA TCA TTC AAG AGC CCA GAA CCC AGG CTC TTT ACT       336
Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110

CCT GAA GAA TTC TTT AGA ATT TTT AAT AGA TCC ATT GAT GCC TTC AAG       384
Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
            115                 120                 125

GAC TTT GTA GTG GCA TCT GAA ACT AGT GAT AGT GTG GTT TCT TCA ACA       432
Asp Phe Val Val Ala Ser Glu Thr Ser Asp Ser Val Val Ser Ser Thr
        130                 135                 140
```

```
TTA AGT CCT GAG AAA GAT TCC AGA GTC AGT GTC ACA AAA CCA TTT ATG      480
Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
    145                 150                 155

TTA CCC CCT GTT GCA GCC                                          498
Leu Pro Pro Val Ala Ala
160                 165
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Met Glu Gly Ile Cys Arg Asn Arg Val Thr Asn Asn Val Lys Asp Val
 -1   1               5                  10                  15

Thr Lys Leu Val Ala Asn Leu Pro Lys Asp Tyr Met Ile Thr Leu Lys
                20                  25                  30

Tyr Val Pro Gly Met Asp Val Leu Pro Ser His Cys Trp Ile Ser Glu
            35                  40                  45

Met Val Val Gln Leu Ser Asp Ser Leu Thr Asp Leu Leu Asp Lys Phe
        50                  55                  60

Ser Asn Ile Ser Glu Gly Leu Ser Asn Tyr Ser Ile Ile Asp Lys Leu
    65                  70                  75

Val Asn Ile Val Asp Asp Leu Val Glu Cys Val Lys Glu Asn Ser Ser
80                  85                  90                  95

Lys Asp Leu Lys Lys Ser Phe Lys Ser Pro Glu Pro Arg Leu Phe Thr
                100                 105                 110

Pro Glu Glu Phe Phe Arg Ile Phe Asn Arg Ser Ile Asp Ala Phe Lys
            115                 120                 125

Asp Phe Val Val Ala Ser Glu Thr Ser Asp Ser Val Val Ser Ser Thr
        130                 135                 140

Leu Ser Pro Glu Lys Asp Ser Arg Val Ser Val Thr Lys Pro Phe Met
    145                 150                 155

Leu Pro Pro Val Ala Ala
160                 165
```

CLAIMS

We claim:

1. A covalently crosslinked biologically active dimer of kit ligand consisting of two monomers, each of the monomers comprising kit ligand amino acid sequences, said dimer being essentially free of monomeric forms of kit ligand and inactive dimers of kit ligand.

2. The dimer according to claim 1, wherein each of the monomers additionally comprise non-kit ligand amino acid sequences.

3. The dimer according to claim 2, wherein the non-kit ligand amino acid sequences are derived from a protein selected from the group consisting of immunoglobulins, C1q and C4bp binding protein.

4. The dimer according to any one of claims 1 to 3, wherein the monomers are covalently crosslinked to one another through the side groups of at least one of their respective amino acids.

5. The dimer according to claim 4, wherein the covalent attachment is a disulfide bond.

6. The dimer according to any one of claims 1 to 3, wherein the monomers are covalently attached directly to one another through an amino acid linker that bonds to the N-terminus of one monomer and the C-terminus of the other monomer.

7. The dimer according to any one of claims 1 to 6, wherein the kit ligand amino acid sequences in each of the monomers is independently selected from the group consisting of kit ligand amino acids 1-138, kit ligand amino acids 1-162, kit ligand amino acids 1-164 and kit ligand amino acids 1-165.

8. The dimer according to claim 4, wherein each of the monomers has an amino acid sequence selected from SEQ ID NO.:2 or SEQ ID NO.:4, and each monomer comprises an intrachain disulfide bond between Cys_4 and Cys_{89} or between Cys_{43} and Cys_{138}.

9. The dimer according to claim 4, wherein the non-kit ligand amino acid sequences are derived from an immunoglobulin heavy chain.

10. The dimer according to claim 10, wherein the monomers are selected from the SEQ ID NO:8, SEQ ID NO:12 or SEQ ID NO:14.

11. The dimer according to claim 4, wherein the KL amino acid sequences in each monomer are selected from SEQ ID NO:18 or SEQ ID NO:20.

12. The dimer according to claim 6, having an amino acid sequence selected from SEQ ID NO:28 or SEQ ID NO:32.

13. The dimer according to claim 4, wherein the KL amino acid sequence in each monomer is independently selected from SEQ ID NO:34 or SEQ ID NO: 36.

14. A covalently crosslinked biologically active dimer of FLT-3/FLK-2 ligand consisting of two monomers, each of the monomers comprising FLT-3/FLK-2 ligand amino acid sequences, said dimer being essentially free of monomeric forms of FLT-3/FLK-2 ligand and inactive dimers of FLT-3/FLK-2 ligand.

15. The dimer according to claim 14, wherein each of the monomers additionally comprise non-FLT-3/FLK-2 ligand amino acid sequences, with the proviso that the nonFLT-3/FLK-2 ligand amino acid sequences are not derived from immunoglobulins.

16. The dimer according to claim 15, wherein the non-FLT-3/FLK-2 ligand amino acid sequences are derived from a protein selected from the group consisting of C1q and C4bp binding protein.

17. The dimer according to any one of claims 14 to 16, wherein the monomers are covalently crosslinked to one another through the side groups of at least one of their respective amino acids.

18. The dimer according to claim 17, wherein the covalent attachment is a disulfide bond.

19. The dimer according to any one of claims 14 to 16, wherein the monomers are covalently attached directly to one another through an amino acid linker that bonds to the N-terminus of one monomer and the C-terminus of the other monomer.

20. The dimer according to any one of claims 14 to 19, wherein the FLT-3/FLK-2 ligand amino acid sequences in each of the monomers is independently selected from the group consisting of FLT-3/FLK-2 ligand amino acids 1-135 and FLT-3/FLK-2 ligand amino acids 1-163.

21. A recombinant DNA molecule characterized by a nucleic acid sequence encoding a fusion protein comprising kit ligand amino acid sequences fused to non-kit ligand amino acid sequences, wherein upon expression of said nucleic acid sequence in a suitable host, said fusion protein forms a covalent crosslinked biologically active dimer of kit ligand as in claim 2.

22. The recombinant DNA molecule according to claim 21, wherein the non-KL amino acid sequences are derived from a protein selected from the group consisting of immunoglobulins, immunoglobulin fragments, C1q and C4bp binding protein.

23. The recombinant DNA molecule according to claim 22, wherein said nucleic acid sequence is selected from SEQ ID NO:7, SEQ ID NO:11 or SEQ ID NO:13.

24. A recombinant DNA molecule characterized by a nucleic acid sequence encoding a polypeptide comprising the formula: KL_1-linker-KL_2, wherein
KL_1 and KL_2 are independently kit ligand amino acid sequences; and
linker is from 3 to 50 independently selected amino acids.

25. The recombinant DNA molecule according to claim 24, wherein said linker comprises the formula:
$(Gly_4\text{-}Ser)_n$, wherein n is an integer from 1 to 9.

26. The recombinant DNA molecule according to claim 25, wherein the nucleic acid sequence is selected from SEQ ID NO:27 and SEQ ID NO:31

27. A host transformed with the recombinant DNA molecule according to any one of claims 21-26.

28. The host according to claim 27 selected from the group consisting of bacteria, yeast, insect, mammalian cells, and transgenic animals.

29. A method for making a covalently crosslinked biologically active dimer of kit ligand comprising the steps of:

(a) transforming or transfecting a suitable host cell with a recombinant DNA molecule characterized by a DNA sequence encoding a polypeptide comprising kit ligand amino acid sequences;

(b) incubating said host cell under condtions which cause expression of said polypeptide;

(c) isolating said polypeptide from contaminant polypeptides which do not contain said kit ligand amino acids;

(d) optionally employing crosslinking means to convert at least a portion of said isolated polypeptide molecules into a covalently crosslinked dimer of kit ligand; and

(e) separating said covalently crosslinked dimer of kit ligand from monomeric forms of kit ligand and from inactive dimers of kit ligand.

30. The method of claim 29 wherein said crosslinking means comprises the steps of:

(a) denaturing said polypeptide; and

(b) refolding the polypeptide at a pH between about 8 and 9.

31. The method of claim 29 wherein said recombinant DNA molecule is selected from a recombinant DNA molecule according to any one of claims 21-26, or a recombinant DNA molecule characterized by a nucleic acid sequence according to any one of SEQ ID NOS: 1, 3, 17, 19, 33 or 35.

32. A pharmaceutically acceptable composition comprising:

(a) a covalently crosslinked dimer of kit ligand according to any one of claims 1-13 in an amount effective for enhancing the proliferative acitivty of hemopoietic cells; and

(b) a pharmaceutically acceptable carrier.

33. A pharmaceutically acceptable composition comprising:

(a) a covalently crosslinked dimer of FLT-3/FLK-2 ligand according to any one of claims 14-20 in an amount effective for enhancing the proliferative activity of hemopoietic cells; and

(b) a pharmaceutically acceptable carrier.

34. A method for enhancing the proliferative activity of hematopoietic cells comprising the step of administering to the cells a composition according to claim 32 or claim 33.

35. A method for desensitizing the mast cells of a patient to be treated with a therapeutic dose of kit ligand which enhances hematopoietic recovery in a patient or mobilizes progenitors or stem cells to the peripheral blood, said method comprising the step of administering to the patient a composition according to claim 32.

36. The method of claim 35 wherein the composition is administered at a dosage of between 0.1 and 25 μg/kg of body weight.

37. The method of claim 35 wherein the composition is administered to the patient between 30 minutes and three hours prior to treatment with the therapeutic dose of kit ligand.

Amino Acid Sequence Alignment of The Soluble Form of Kit Ligand from Human, Mouse, and Rat

```
     1                                                        25
Hu   E G I C R N R V T N N V K D V T K L V A N L P K D
Mu   K E I C G N P V T D N V K D I T K L V A N L P N D
Ra   Q E I C R N P V T D N V K D I T K L V A N L P N D

                                                        50
Hu   Y M I T L K Y V P G M D V L P S H C W I S E M V V
Mu   Y M I T L N Y V A G M D V L P S H C W L R D M V I
Ra   Y M I T L N Y V A G M D V L P S H C W L R D M V T

                                                        75
Hu   Q L S D S L T D L L D K F S H I S E G L S N Y S I
Mu   Q L S L S L T T L L D K F S H I S E G L S H Y S I
Ra   H L S V S L T T L L D K F S H I S E G L S N Y S I

                                                       100
Hu   I D K L V N I V D D L V E C V K E H S S K D L K K
Mu   I D K L G K I V D D L V L C M E E H A P K N I K E
Ra   I D K L G K I V D D L V A C M E E N A P K N V K E

                                                       125
Hu   S F K S P E P R L F T P E E F F R I F H R S I D A
Mu   S P K R P E T R S F T P E E F F S I F H R S I D A
Ra   S L K K P E T R N F T P E E F F S I F N R S I D A

                                                       150
Hu   F K D F V V A S E T S D C V V S S T L S P E K D S
Mu   F K D F M V A S D T S D C V L S S T L G P E K D S
Ra   F K D F M V A S D T S D C V L S S T L G P E K D S

                                                  165
Hu   R V S V T K P F M L P P V A A
Mu   R V S V T K P F M L P P V A A
Ra   R V S V T K P F M L P P V A A
```

FIGURE 1

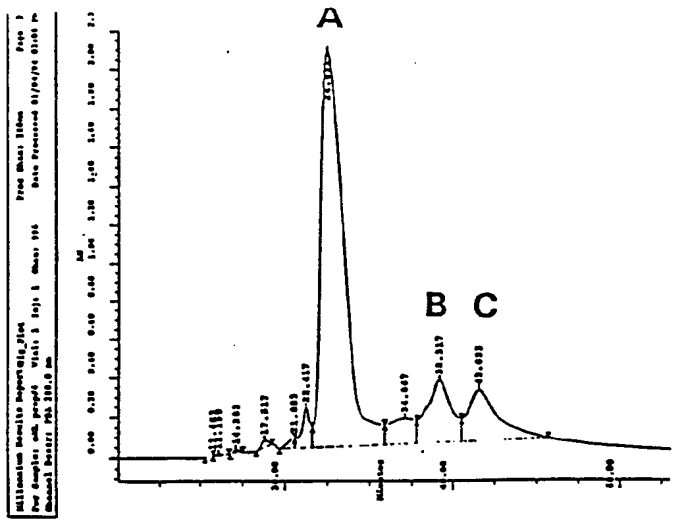


FIGURE 2A

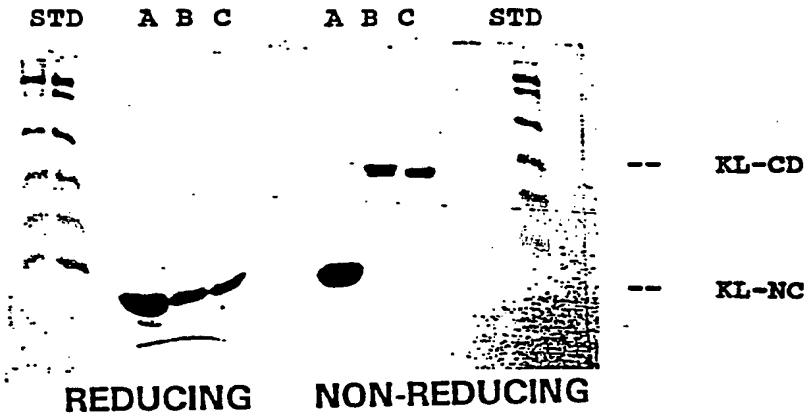


FIGURE 2B

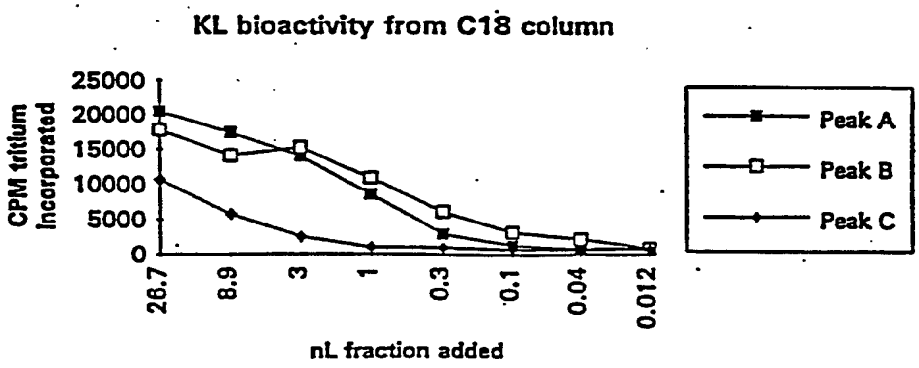


FIGURE 2C

FIGURE 3A

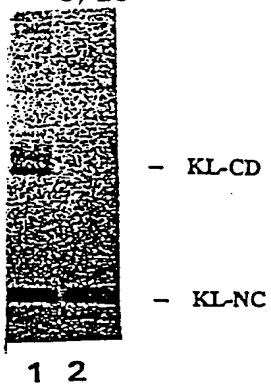


FIGURE 3B

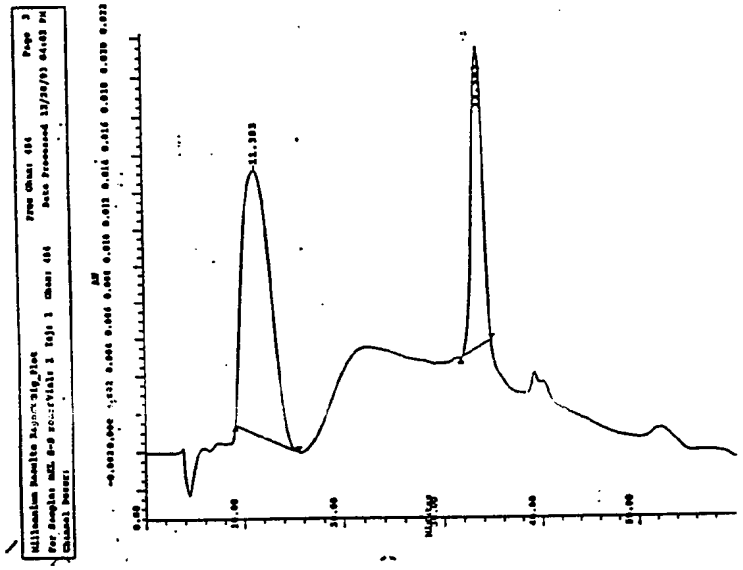
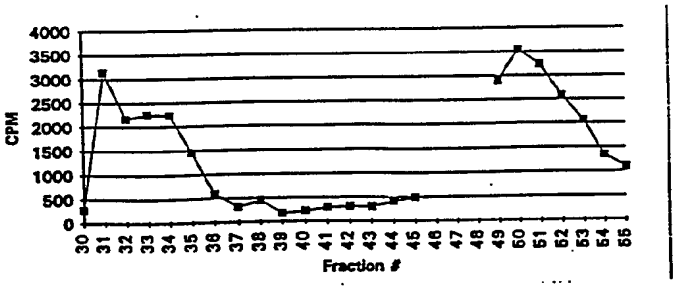


FIGURE 3C



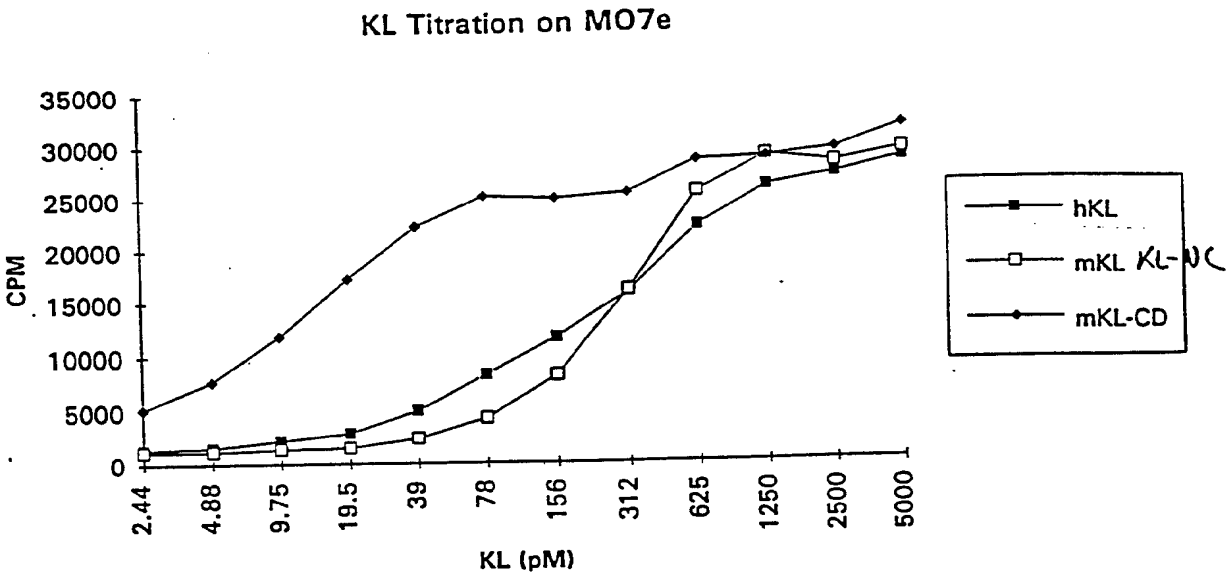


FIGURE 4

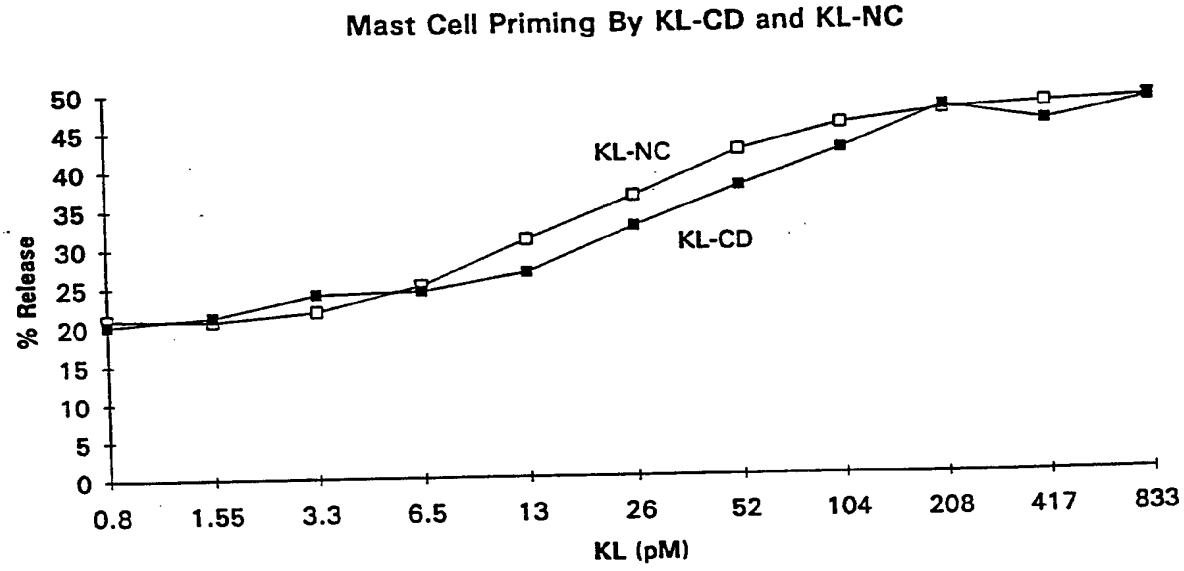


FIGURE 5

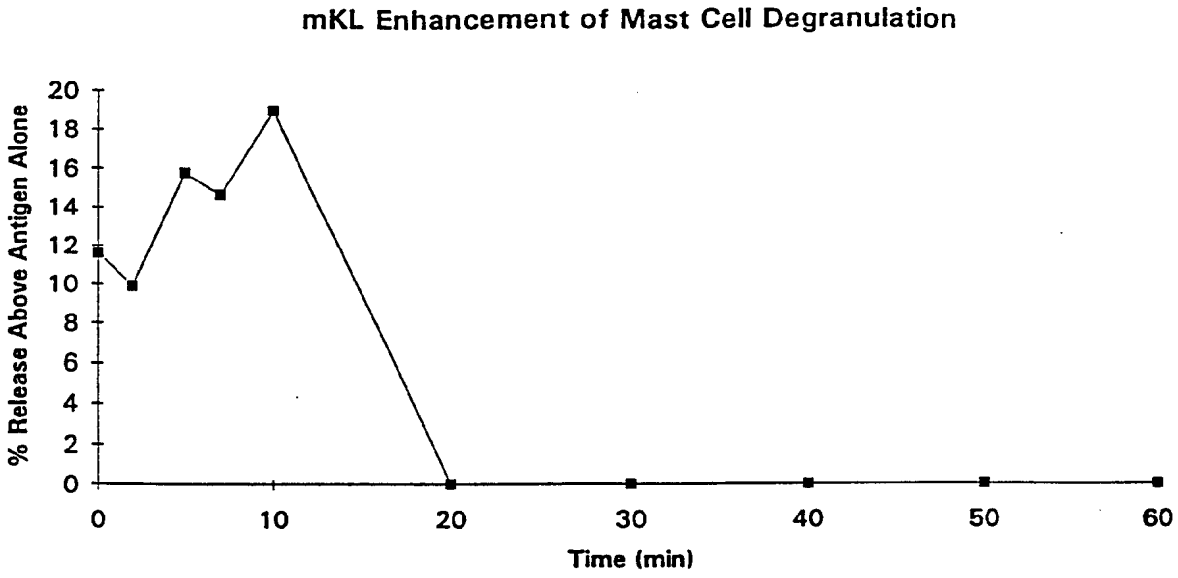
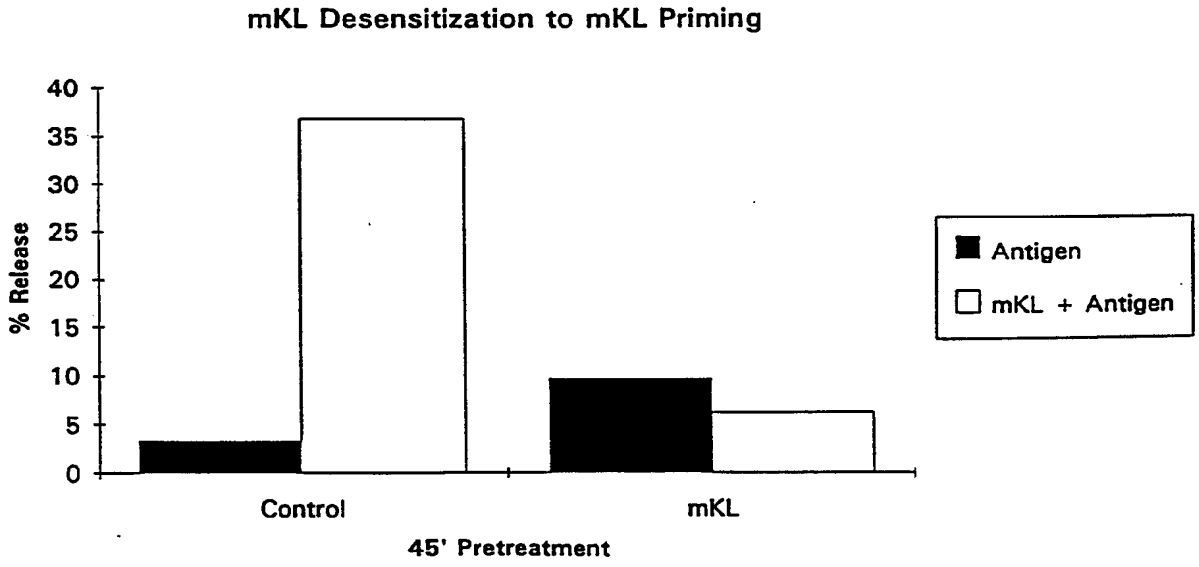
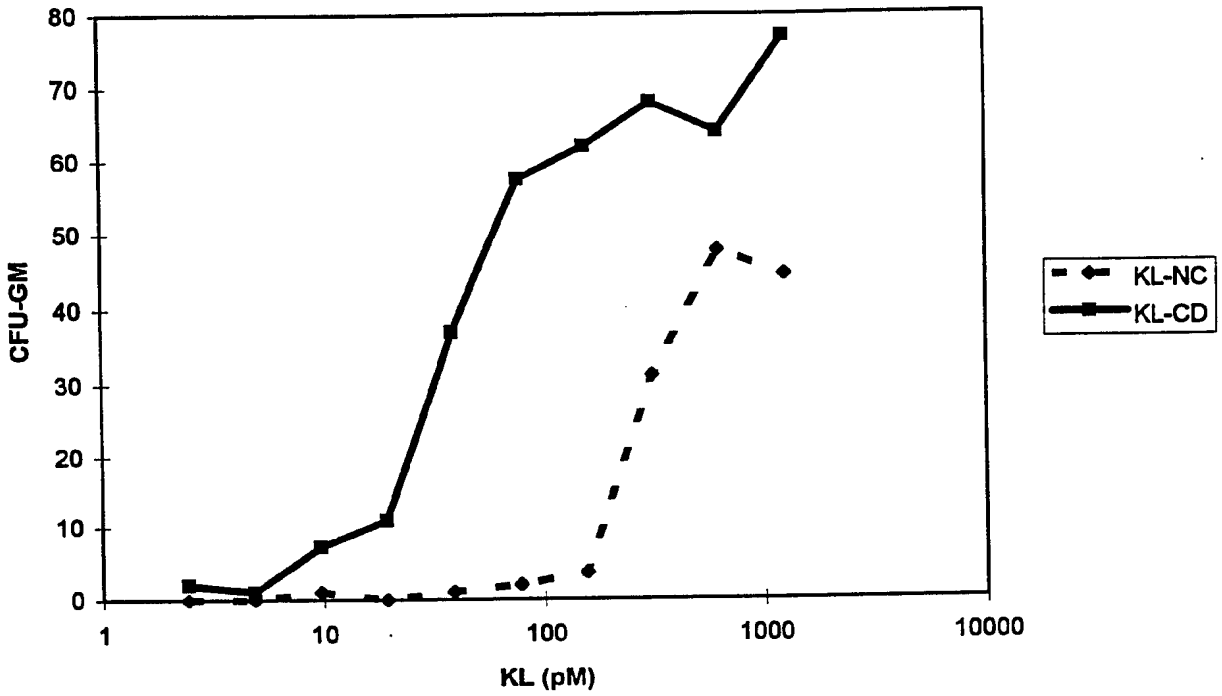

mKL Enhancement of Mast Cell Degranulation
% Release Above Antigen Alone
20
18
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
Time (min)

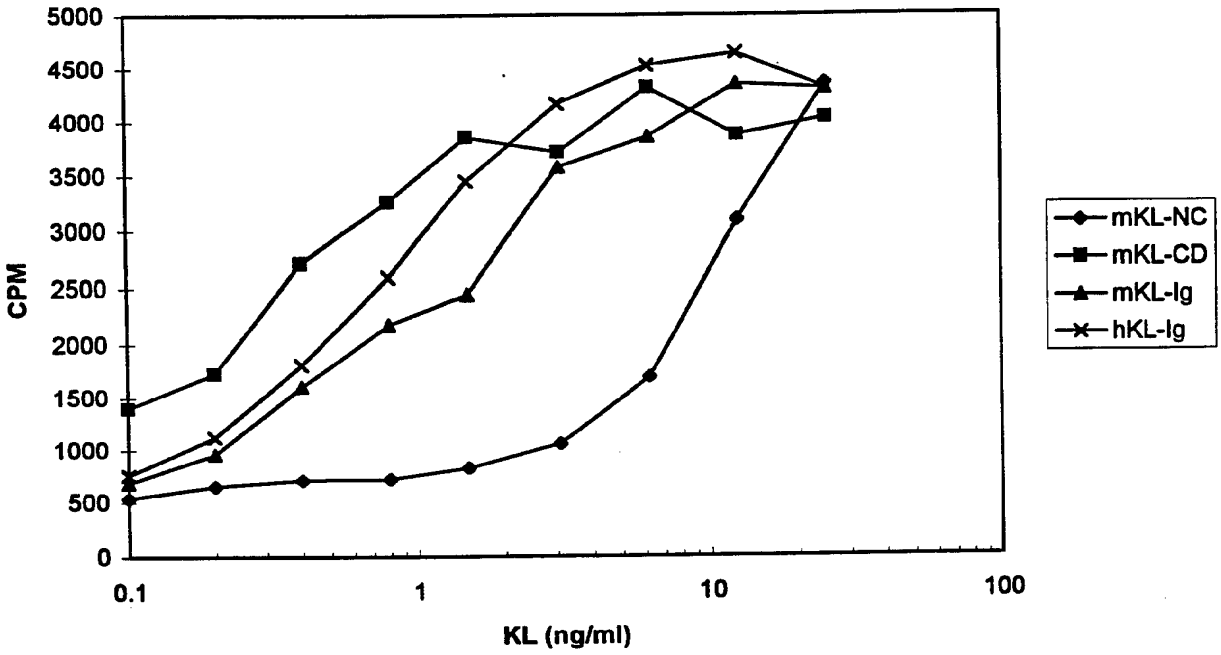
FIGURE 4


mKL Desensitization to mKL Priming
% Release
40
35
30
25
20
15
10
5
0
Control
mKL
45' Pretreatment
Antigen
mKL + Antigen

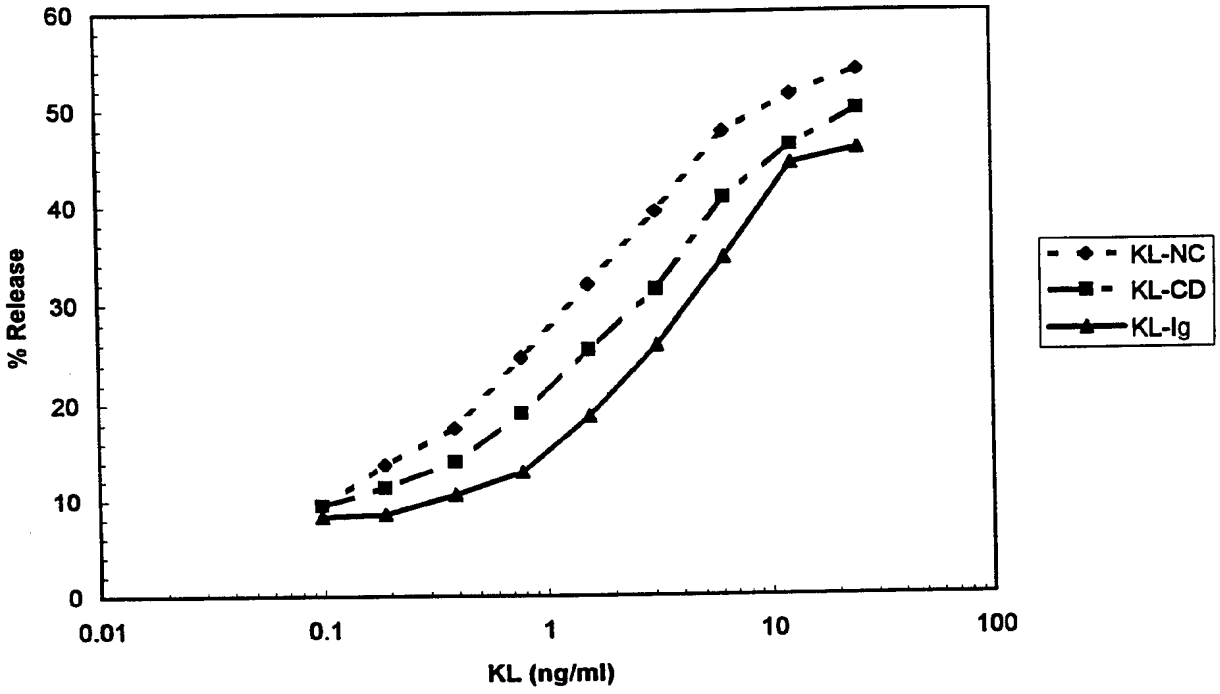
Murine CFU-GM Assay

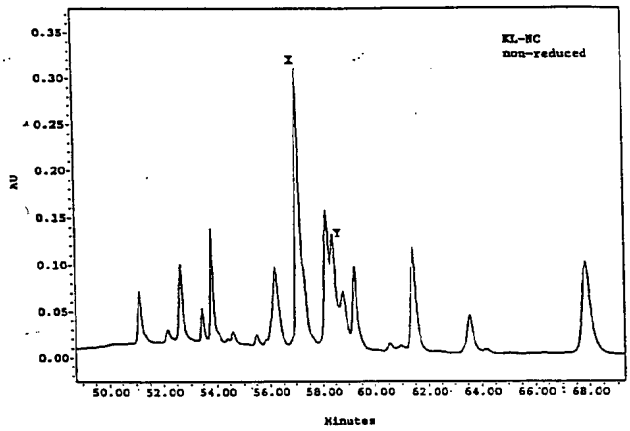


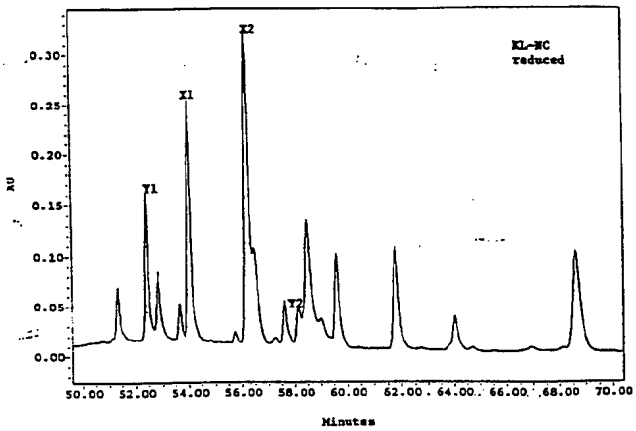
mKL-NC, mKL-CD, mKL-Ig, hKL-Ig Induced Proliferation of the MO7e Cell Line

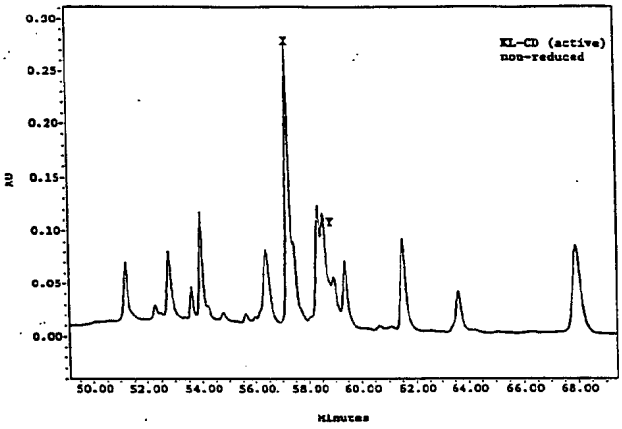


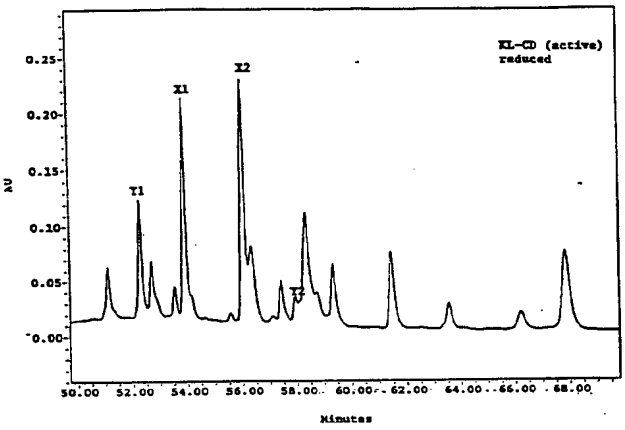
KL-Ig, CD and NC Priming of IgE Induced Degranulation of BMMC

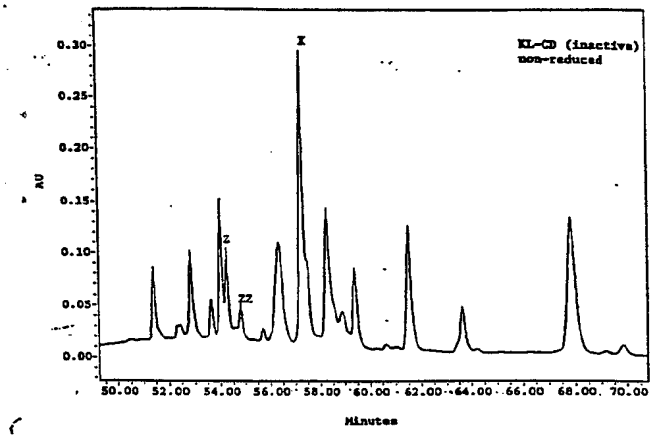


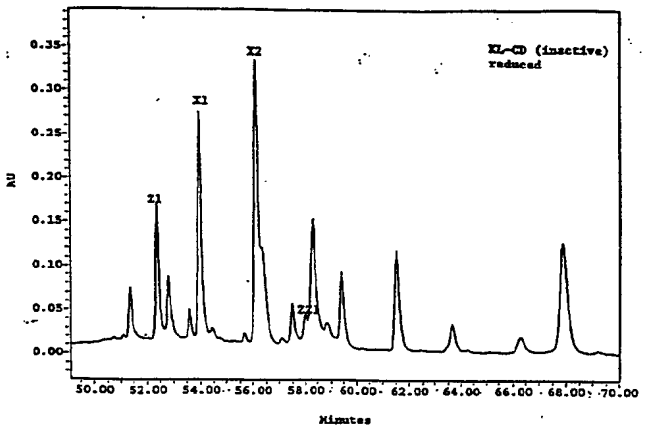

KL-HC
non-reduced
X
Y
AU
Minutes


KL-HC
reduced
Y1
X1
X2
Y2
AU
Minutes

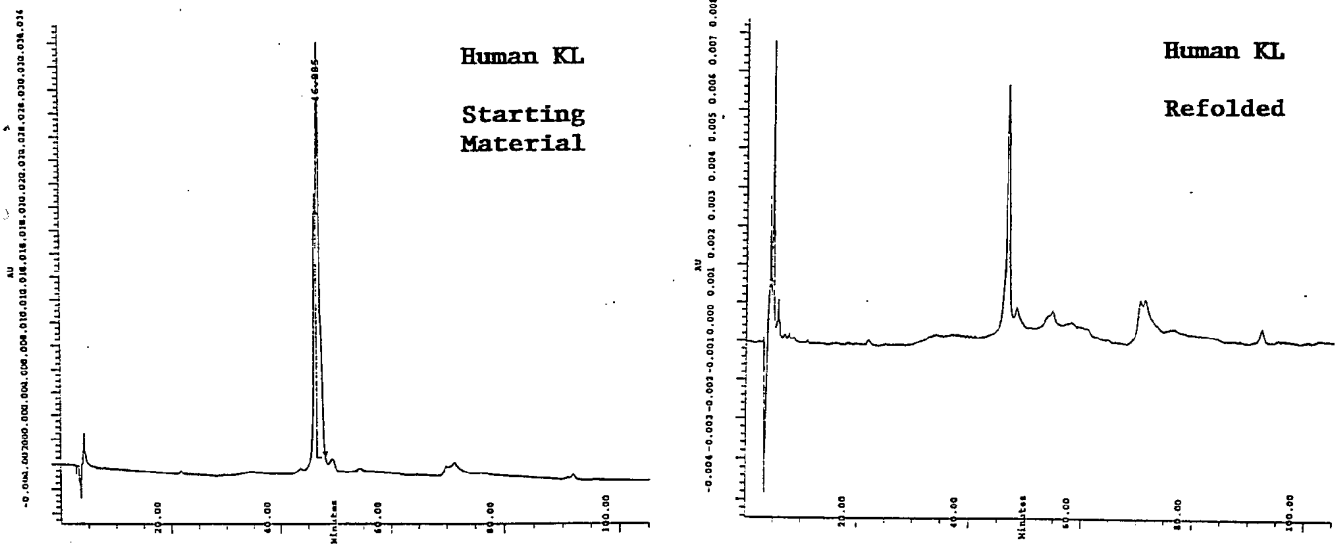

KL-CD (active)
non-reduced
X
Y
AU
Minutes


KL-CD (active)
reduced
Y1
X1
X2
Y2
AU
Minutes

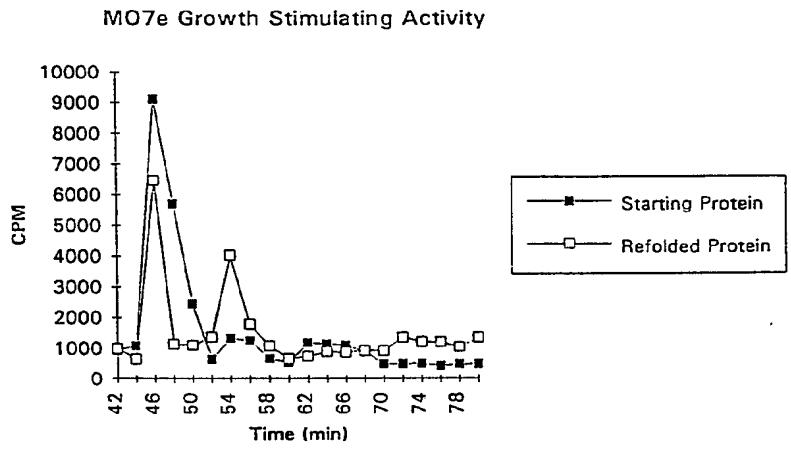

KL-CD (inactive)
non-reduced
X
Z
Z2
AU
Minutes


KL-CD (inactive)
reduced
Z1
X1
X2
Z2
AU
Minutes

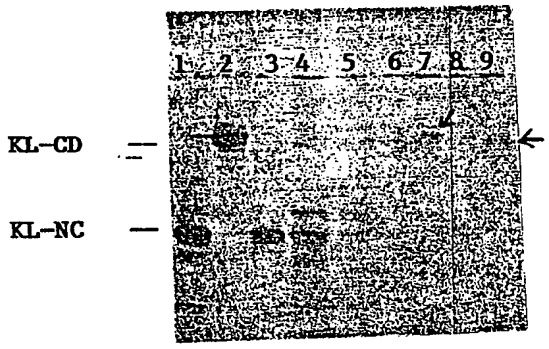
A

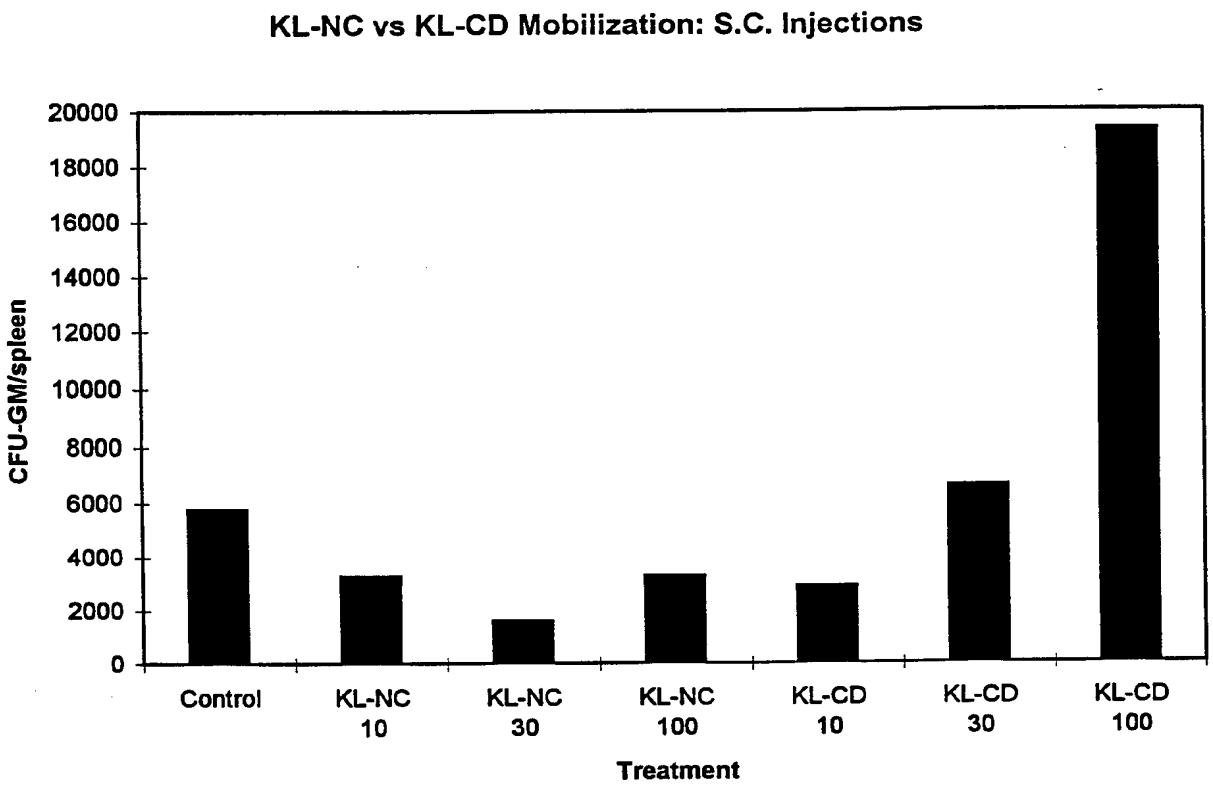

Human KL
Starting Material
Human KL
Refolded

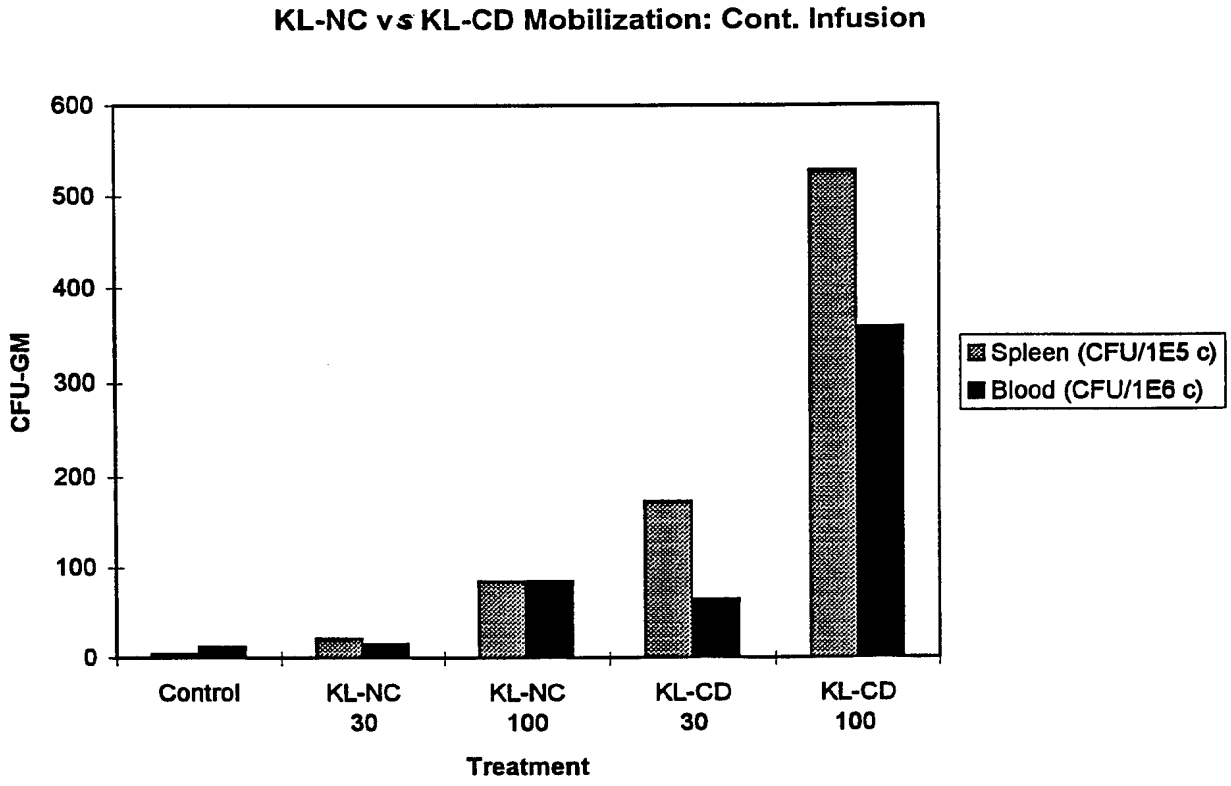
B

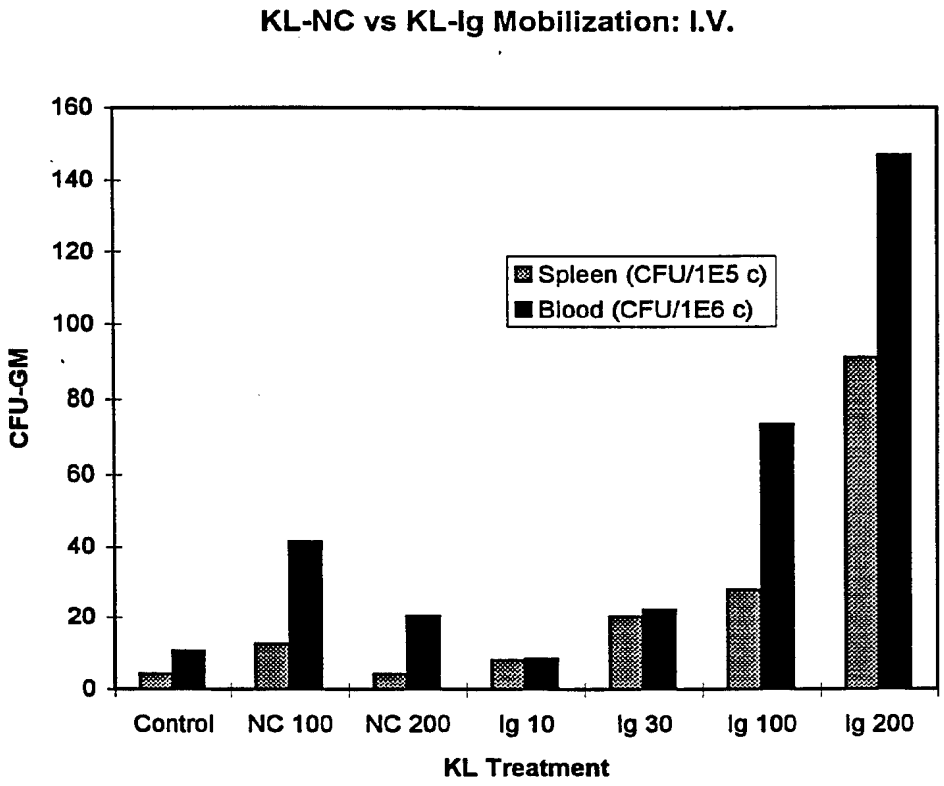

MO7e Growth Stimulating Activity
CPM
Time (min)
Starting Protein
Refolded Protein

C


1 2 3 4 5 6 7 8 9
KL-CD
KL-NC


KL-NC vs KL-CD Mobilization: S.C. Injections
CFU-GM/spleen
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Control
KL-NC 10
KL-NC 30
KL-NC 100
KL-CD 10
KL-CD 30
KL-CD 100
Treatment


KL-NC vs KL-CD Mobilization: Cont. Infusion
600
500
400
300
200
100
0
CFU-GM
Control
KL-NC 30
KL-NC 100
KL-CD 30
KL-CD 100
Treatment
Spleen (CFU/1E5 c)
Blood (CFU/1E6 c)


KL-NC vs KL-Ig Mobilization: I.V.
160
140
120
100
80
60
40
20
0
CFU-GM
Spleen (CFU/1E5 c)
Blood (CFU/1E6 c)
Control
NC 100
NC 200
Ig 10
Ig 30
Ig 100
Ig 200
KL Treatment

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03866

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6,-69.1, 69.5, 69.7, 172.3, 240.2, 320.1; 514/2, 12; 530/350, 351; 536/23.4, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS and DIALOG (files 5, 155, 351, 357, 358) search terms: kit ligand, stem cell factor, KL, flt-3, flk-2, receptor, fusion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y ---- A	WO, A, 91/05795 (AMGEN INC.) 02 May 1991, see Example 10, especially page 106.	1, 4-5, 7, 29-30 ---------- 8, 11, 13, 31-32 ---------- 2-3, 6, 9-10, 12, 21-27
X,P ------ Y,P ------ A,P	WO, A, 94/28391 (IMMUNEX CORPORATION) 08 December 1994, see abstract, claims, and figures.	14, 17-19, 33 ---------- 20 ---------- 15-16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
12 MAY 1995

Date of mailing of the international search report
03 JUL 1995

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US95/03866

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

—

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-27 and 29-33

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/03866

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 37/02, 48/00; C07K 13/00, 15/28; C12N 15/12, 15/64; C07H 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/6, 69.1, 69.5, 69.7, 172.3, 240.2, 320.1; 514/2, 12; 530/350, 351; 536/23.4, 23.5

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I.Claims 1-13, 21-27, and 29-32, drawn to polynucleotides encoding kit ligand monomers and dimers, the proteins encoded, methods of making the proteins, classified in at least Class 536, subclass 23.5, for example.
II.Claims 14-20 and 33, drawn to FLT-3/FLK-2 ligand dimer, classified in at least Class 530, subclass 350, for example.
III.Claim 28, drawn to a transgenic animal, classified in at least Class 800, subclass 2, for example.
IV.Claims 34-37, drawn to methods of treatment, classified in at least Class 514, subclass 12, for example.

The claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. Groups I-III are drawn to structurally different products and Groups I and IV are drawn to methods with different goals, method steps, and starting materials, wherein none of the groups share the same or corresponding "special technical feature". Note that PCT Rule 13 does not provide for multiple products or methods within a single application.